MYCOTAXON

THE INTERNATIONAL JOURNAL OF FUNGAL TAXONOMY & NOMENCLATURE

VOLUME 129(1) JULY–SEPTEMBER 2014

Ypsilomyces elegans **gen. & sp. nov.**
(Almeida & Gusmão— FIG. 2, p. 184)
DAVI AUGUSTO CARNEIRO DE ALMEIDA, *artist*

ISSN (PRINT) 0093-4666 http://dx.doi.org/10.5248/129(1) ISSN (ONLINE) 2154-8889
MYXNAE 129(1): 1–214 (2014)

Editorial Advisory Board

Scott A. Redhead (2010–2015), Chair
Ottawa, Ontario, Canada

Wen-Ying Zhuang (2003-2014), Past Chair
Beijing, China

Sabine Huhndorf (2011–2016)
Chicago, Illinois, U.S.A.

Peter Buchanan (2011–2017)
Auckland, New Zealand

Brandon Matheny (2013-2018)
Knoxville, Tennessee, U.S.A.

Karen Hansen (2014-2019)
Stockholm, Sweden

Published by

Mycotaxon, Ltd.

p.o. box 264, Ithaca, NY 14581-0264, USA

www.mycotaxon.com & www.ingentaconnect.com/content/mtax/mt

© Mycotaxon, Ltd, 2014

MYCOTAXON

THE INTERNATIONAL JOURNAL OF FUNGAL TAXONOMY & NOMENCLATURE

VOLUME 129(1)

OCTOBER–DECEMBER 2014

EDITOR-IN-CHIEF

LORELEI L. NORVELL

editor@mycotaxon.com

Pacific Northwest Mycology Service
6720 NW Skyline Boulevard
Portland, Oregon 97229-1309 USA

NOMENCLATURE EDITOR

SHAUN R. PENNYCOOK

PennycookS@LandcareResearch.co.nz

Manaaki Whenua Landcare Research
Auckland, New Zealand

BOOK REVIEW EDITOR

ELSE C. VELLINGA

bookreviews@mycotaxon.com

861 Keeler Avenue
Berkeley CA 94708 U.S.A.

CONSISTING OF I–X + 214 PAGES INCLUDING FIGURES

ISSN 0093-4666 (PRINT) http://dx.doi.org/10.5248/129(1).cvr ISSN 2154-8889 (ONLINE)

© 2014. MYCOTAXON, LTD.

MYCOTAXON

VOLUME ONE HUNDRED TWENTY-NINE(1) — TABLE OF CONTENTS

Cover section

Errata vi
Reviewers vii
Submission procedures viii
From the Editor ix

Research articles

Beltraniella species associated with leaf litter of the Atlantic Forest in southern Bahia, Brazil — Marcos Vinícius Oliveira Dos Santos, Flávia Rodrigues Barbosa, Dilze Maria Argôlo Magalhães, Edna Dora Martins Newman Luz, & José Luiz Bezerra 1

Neofomitella polyzonata gen. et sp. nov., and *N. fumosipora* and *N. rhodophaea* transferred from *Fomitella* — Hai-Jiao Li, Xing-Chun Li, Josef Vlasák, & Yu-Cheng Dai 7

A new species of *Uromyces* from Turkey — Zeliha Bahcecioglu 21

Cladosporium species from hypersaline environments as endophytes in leaves of *Cocos nucifera* and *Vitis labrusca* — Rafael José Vilela de Oliveira, Thaís Emanuelle Feijó de Lima, Gladstone Alves da Silva, & Maria Auxiliadora de Queiroz Cavalcanti 25

Aschersonia narathiwatensis sp. nov. from southern Thailand — Suchada Mongkolsamrit, Artit Khonsanit, Wasana Noisripoom, Pagmadulam Baldorj, & J. Jennifer Luangsa-ard 33

South Florida microfungi: *Linkosia longirostrata*, a new hyphomycete on paurotis palm — Gregorio Delgado 41

Taxonomy and phylogeny of *Heterobasidion* in South Korea — Yeongseon Jang, Seokyoon Jang, Young Woon Lim, Changmu Kim, & Jae-Jin Kim 47

Two new combinations and a new record of *Zasmidium* from China — Fengyan Zhai, W.H. Hsieh, Yingjie Liu, & Y.L. Guo 57

New reports of *Gymnopus* from Pakistan based on ITS sequences — M. Saba & A.N. Khalid 63

First report of *Callistosporium luteoolivaceum* from Western Himalaya, Pakistan — M. Saba & A.N. Khalid 73

Gastroboletus thibetanus: a new species from China — Shu-Rong Wang, Qi Wang, De-Li Wang, & Yu Li 79

Biogeographical patterns in pyrenomycetous fungi and their taxonomy. 4. *Hypoxylon* and the southern United States
Larissa N. Vasilyeva & Steven L. Stephenson 85

Rhizophagus natalensis, a new species in the *Glomeromycota*
Janusz Błaszkowski, Gerard Chwat, Anna Góralska, & Bruno T. Goto 97

A new species of *Nawawia* from Malaysia, with a synopsis of the genus Teik-Khiang Goh, Wai-Yip Lau, & Kah-Cheng Teo 109

The *Entolomataceae* of the Pakaraima Mountains of Guyana 6: ten new species and a new combination in *Nolanea* Terry W. Henkel, M. Catherine Aime, David L. Largent, & Timothy J. Baroni 119

Two new species of *Xylaria* and *X. diminuta* new to China
Gu Huang, Lin Guo, & Na Liu 149

Erysiphe magnoliicola, a new powdery mildew on *Magnolia*
Sung-Eun Cho, Susumu Takamatsu, Jamjan Meeboon, & Hyeon-Dong Shin 153

Phoma recepii sp. nov. from the *Caloplaca cerina* group in Turkey
Mehmet Gökhan Halici, Mehmet Candan, Mithat Güllü, & Ahmet Özcan 163

Geastrum from the Atlantic Forest in northeast Brazil — new records for Brazil Julieth de Oliveira Sousa, Bianca Denise Barbosa da Silva, & Iuri Goulart Baseia 169

Ypsilomyces, a new thallic genus of conidial fungi from the semi-arid Caatinga biome of Brazil Davi Augusto Carneiro de Almeida & Luís Fernando Pascholati Gusmão 181

Agaricus taeniatus sp. nov., a new member of *Agaricus* sect. *Bivelares* from northwest China Sai-Fei Li, Ya-Li Xi, Cai-Xia Qi, Qian-Qian Liang, Sheng-Long Wei, Guo-Jie Li, Dong Zhao, Shao-Jie Li, & Hua-An Wen 187

Macrolepiota distribution extends to the montane temperate forests of Pakistan Muhammad Fiaz, Sana Jabeen, Amna Imran, Habib Ahmad, & Abdul Nasir Khalid 197

Hyphodontia dhingrae sp. nov. from India
Samita, S.K. Sanyal, & G.S. Dhingra 209

Nomenclatural novelties and typifications proposed in Mycotaxon 129(1) 213

Errata from previous volumes

Volume 128

p.99, line 11	for: *Penicillium citreonigrum*	read: *Penicillium chrysogenum*
p.100, Table 2, line 9	for: MTCC 3019	read: MTCC 3017
p.100, Table 2, line 11	for: *Penicillium citreonigrum*	read: *Penicillium chrysogenum*
p.114, bottom line	for: C, D = 50 µm	read: C, D, F = 50 µm

publication date for volume one hundred twenty-eight
MYCOTAXON *for* April–June, volume 128 (i–ix + 1–208)
was issued on August 21, 2014

Reviewers — volume one hundred twenty-nine (one)

The Editors express their appreciation to the following individuals who have, prior to acceptance for publication, reviewed one or more of the papers prepared for this issue of Mycotaxon 129.

Vladimir Antonín
J.L. Azevedo
José Luiz Bezerra
Wolfgang von Brackel
Uwe Braun
Mehmet Candan
R.F. Castañeda-Ruiz
Michael Castellano
Santiago Chacón
Johannes C. Coetzee
Yu-Cheng Dai
Kanad Das
Antonio Hernández Gutiérrez
Ian Robert Hall
Nils Hallenberg
Richard A. Humber
Bryce Kendrick
Kerry Knudsen
T.K. Arun Kumar
David P. Lewis
De-Wei Li
Shuyan Liu
Hayato Masuya
Juan Luis Mata
Wieslaw Mulenko
Lorelei L. Norvell
Fritz Oehl
Clark L. Ovrebo
Shaun R. Pennycook
Ronald H. Petersen
Amy Y. Rossman
Álvaro Figueredo Dos Santos
B.M. Sharma
Gladstone Alves da Silva
Michal Tomšovský
Clement Tsui
Larissa Vasilyeva
Else C. Vellinga
Nadja Santos Vitória
Felipe Wartchow
Anthony J.S. Whalley
Bayram Yıldız
Rui-Lin Zhao
Li-Wei Zhou

Four steps to successful Mycotaxon publication in 2014

Prospective Mycotaxon authors should download instructions PDF, review and submission forms, and other helpful templates by clicking the 'file download page' link on our Instructions to Authors page before preparing their manuscript. Below is a summary of our '4-step' publication process.

1—Peer review: Email formatted text and illustration files with a 2014 Mycotaxon Reviewer Comments Form to 2–3 experts for peer review. Authors should (i) ask peer reviewers to return revisions and comment forms to both authors and *Editor-in-Chief* <editor@mycotaxon.com> and (ii) follow reviewer suggestions **before** sending revised files to the *Nomenclature Editor* for nomenclatural review.

2—Nomenclatural review: Email text files (with tables & captions but **no** artwork) to the *Nomenclature Editor* <PennycookS@LandcareResearch.co.nz> for accession and pre-submission review. The Email message *MUST* include 'Mycotaxon' on the subject line **and** all peer reviewer names+Email addresses in the message. The Nomenclature Editor will assign accession numbers and return annotated files with a list of needed corrections to the authors and *Editor-in-Chief.*

3—Final submission: After consulting experts and revising manuscripts as needed, send the (i) completed 2014 Mycotaxon submission form; (ii) separate text files for main text, tables, and legends; and (iii) art files to the *Editor-in-Chief* <editor@mycotaxon.com>. Only text and image files formatted for immediate approval should be sent at this time. The *Editor-in-Chief* usually acknowledges manuscripts and thanks expert reviewers within two weeks, but please wait at least 14 days before sending a follow-up query (without attachments); this helps us keep Email traffic to a minimum during Mycotaxon publication deadlines or temporary closures of the editorial office.

4—Final editorial review & press preparation: Files not ready for publication will be rejected or returned to authors for further revision; the *Editor-in-Chief* gives tentatvely approved manuscripts a final grammatical and scientific review before converting all files into publishable format. The PDF proof, bibliographic citation, and nomenclatural entries are sent to all coauthors for final inspection prior to publication. After PDF conversion, the *Editor-in-Chief* corrects only processing or editorial errors prior to publication but will list corrections of author errors in the Errata of a subsequent volume for no charge. Authors are expected to arrange payment of page charges and optional open access fees with the *Business Manager* <subscriptions@mycotaxon.com> at this time.

Mycotaxon ltd— www.mycotaxon.com

The Mycotaxon *Webmaster* <mycotaxon@gmail.com> posts general and subscription information, important announcements, and author forms and templates on the official Mycotaxon site. The server also hosts the regional mycobiota webpage for free download of distributional annotated species lists.

Mycotaxon online— www.ingentaconnect.com/content/mtax/mt

Mycotaxon publishes quarterly. Both open access and subscription articles are offered.

From the Editor-in-Chief

The 'issue' issue — Mycotaxon has always prided itself on being able to deliver complete volumes quarterly, making it an exception in the world of periodical journals. We based our nomenclature on the fact that any individual publication containing over 400 pages merited the name 'volume.' In the past year or so, however, what the editorial office calls a 'perfect storm' of misfortune — bad health, the need for page charges, and proliferation of competing online alternatives — has produced a temporary publications bottleneck leading to fewer pages. In an effort to speed up our quarterly delivery, we have decided to publish every three months regardless of size, assigning issue numbers to smaller publications as needed. Welcome, therefore, to Mycotaxon 129(1). We hope to deliver the remainder of this volume [Mycotaxon 129(2)] shortly!

Instructions to Authors: simplified! — Instructions are the bane of author and editor alike. Authors too often find them picky and/or incomprehensible, while editors despair because few follow the guidelines that they have spent too much time fine-tuning. At the moment, we all agree that Mycotaxon instructions are too long to help anyone. The blame for this rests (alas) squarely on my editorial shoulders, which are now straightening to chop, cut, and simplify so that our authors can spend far less time changing font sizes and margins and much more time on research. Stay tuned for further developments.

Mycotaxon 129(1) contains 22 papers by 82 authors (representing 15 countries) and revised by 45 expert reviewers.

Within its pages are two new genera (*Neofomitella* from China and *Ypsilomyces* from Brazil) and 30 species new to science representing *Agaricus, Gastroboletus,* and *Xylaria* from China; *Aschersonia* from Thailand; *Erysiphe* from South Korea and Japan; *Hyphodontia* from India; *Hypoxylon* and *Linkosia* from the U.S.A.; *Nawawia* from Thailand; *Nolanea* from Guyana; *Phoma* and *Uromyces* from Turkey; and *Rhizophagus* from Brazil.

In addition to new combinations in *Neofomitella* and *Zasmidium*, we also offer range extensions of *Callistosporium, Gymnopus,* and *Macrolepiota* species to Pakistan and *Geastrum* species to Brazil as well as conclusions from an excellent taxonomic and phylogenetic study of *Heterobasidion* in South Korea.

Warm regards,

Lorelei L. Norvell (*Editor-in-Chief*)
11 November 2014

ISSN (print) 0093-4666 © 2014. Mycotaxon, Ltd. ISSN (online) 2154-8889

MYCOTAXON

http://dx.doi.org/10.5248/129.1

Volume 129(1), pp. 1–6 *July–September 2014*

Beltraniella species associated with leaf litter of the Atlantic Forest in southern Bahia, Brazil

Marcos Vinícius Oliveira dos Santos[1*], Flávia Rodrigues Barbosa[2], Dilze Maria Argôlo Magalhães[3], Edna Dora Martins Newman Luz[3], & José Luiz Bezerra[1,4]

[1]*Departamento de Micologia, Universidade Federal de Pernambuco, Av. Prof. Nelson Chaves, s/n°, Recife, PE, 50670-901, Brasil*

[2]*Instituto de Ciências Naturais, Humanas e Sociais, Universidade Federal de Mato Grosso, Avenida Alexandre Ferronato, 1200, Setor Industrial, Sinop, MT, 78557-267, Brasil*

[3]*Setor de Fitopatologia, Centro de Pesquisas do Cacau, Comissão Executiva do Plano da Lavoura Cacaueira, Rod. Ilhéus-Itabuna, km 22, Ilhéus, BA, 45662-000, Brasil*

[4]*Centro de Ciências Agrárias e Biológicas, Universidade Federal do Recôncavo da Bahia, Rua Rui Barbosa, 710, Cruz das Almas, BA, 44.380-00, Brasil*

* Correspondence to: *marcosvos@ymail.com*

Abstract — Two species of *Beltraniella*, *B. botryospora* and *B. portoricensis*, were found to be associated with the leaf litter of representative plants of the Atlantic Forest (*Inga thibaudiana*, *Myrcia splendens*, *Pera glabrata*) in the Reserva Biológica de Una, municipality of Una, Bahia State, Brazil. A description and illustration are provided for *B. botryospora,* reported here for the first time from the Americas. A key to *Beltraniella* species cataloged in Brazil is also presented.

Key words — conidial fungi, taxonomy, *Fabaceae*, *Myrtaceae*, *Peraceae*

Introduction

Beltraniella was proposed in 1952 by Subramanian with the type species *B. odinae* Subram., found in India on leaf litter of *Odina wodier* Roxb. (Subramanian 1952). The genus is characterized by setiform conidiophores and polyblastic sympodial denticulate conidiogenous cells. Conidia are turbinate or biconic and often caudate (Ellis 1971).

Beltraniella species are typically found on decomposing fallen leaves on the ground and other natural substrates (Polishook et al. 1996, Marques et al. 2007, Shirouzu et al. 2009, Magalhães et al. 2011) in tropical and subtropical

regions (Kirk 1981). Some species correspond to the anamorphic stage of the ascomycetes *Pseudomassaria* Jacz. or *Leiosphaerella* Höhn. (Kirk et al. 2008).

Currently, approximately 20 species are described in *Beltraniella* (Shirouzu et al. 2010, Priya et al. 2011), of which four have been cataloged in Brazil: *B. amoena* R.F. Castañeda et al., *B. japonica* Matsush., *B. portoricensis,* and *B. fertilis* Heredia et al. (Gusmão et al. 2001, Marques et al. 2007, Magalhães et al. 2011). *Beltraniella portoricensis* and *B. fertilis* have been previously listed as associated with plants endemic to the Atlantic Forest (Magalhães et al. 2011). The present study aimed to identify *Beltraniella* species associated with the leaf litter from three common representative plant species from the Atlantic Forest in the Reserva Biológica de Una, municipality of Una, Bahia State, Brazil.

Materials & methods

The materials were collected in September 2011 and April 2012 in the Reserva Biológica de Una (an Atlantic Forest Conservation Unit), municipality of Una, Bahia State, Brazil. Five specimens of three representative Atlantic Forest tree species were marked and identified in the field as *Inga thibaudiana* DC. (*Fabaceae*), *Myrcia splendens* (Sw.) DC. (*Myrtaceae*), and *Pera glabrata* (Schott) Poepp. ex Baill. (*Peraceae*). The leaf litter of these trees was collected at different stages of decomposition, stored in Kraft paper bags, and sent to the laboratory of Fungal Diversity of the Centro de Pesquisas do Cacau (CEPEC), Comissão Executiva do Plano da Lavoura Cacaueira (CEPLAC), Ilhéus, Bahia State, Brazil.

In the laboratory, the samples were placed in pre-perforated plastic containers and washed gently for one hour in running water so that the water did not hit them directly, thus allowing impurities to be removed. The washed samples were placed in moist chambers, observed after 72 h with a stereomicroscope, and monitored periodically for 30 days. The reproductive structures of the microfungi were removed with a histological needle and placed in a permanent mounting medium containing polyvinyl-lacto-glycerol (PVLG) resin (Silva & Grandi 2011).

The prepared fungal slides were observed using a light microscope, and the species were identified by morphological comparison with specific literature (Ellis 1971, Seifert et al. 2011). Photomicrographs were taken with a Sony Cyber-shot 16.2 megapixel digital camera. Subsequently, the permanent slides were deposited in the Mycological Collection of CEPEC.

Taxonomy

Beltraniella botryospora Shirouzu & Tokum., Fungal Diversity 43: 88 (2010) Plate 1

Colonies amphigenous, effused, dark-brown. Mycelium immersed in the substrate. Setae subulate, arising from lobed basal cells, single, erect, straight or slightly curved, simple, septate, verrucose, dark-brown, 112–380 × 4–8 µm. Conidiophores macronematous, mononematous, with two forms: long conidiophores arising from lobed basal cells, setiform, single, erect, straight

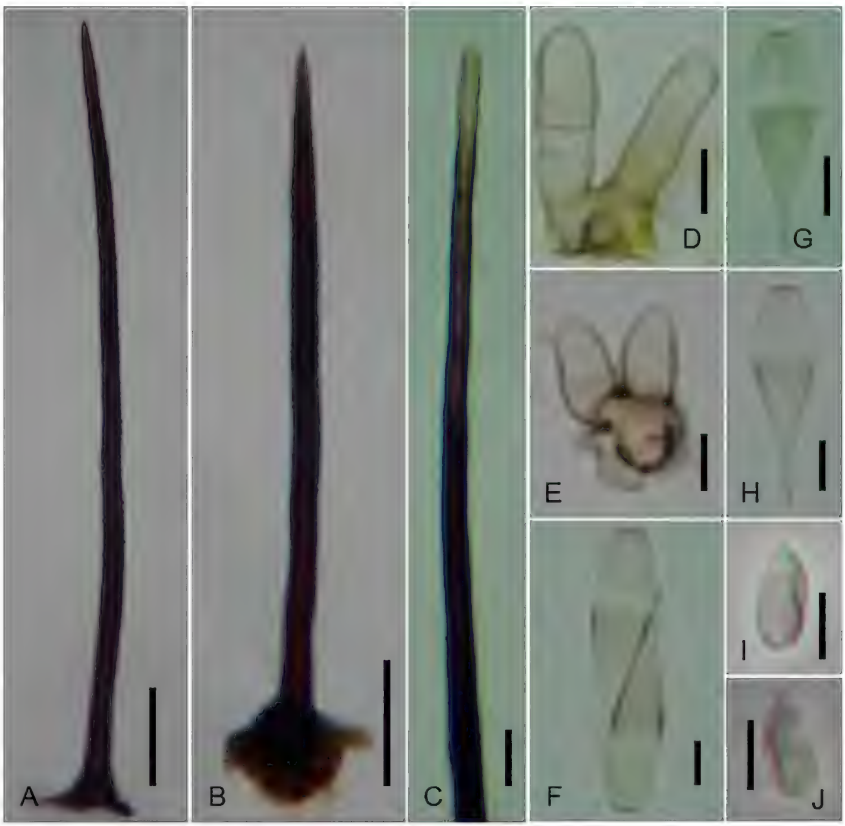


Plate 1. *Beltraniella botryospora*. A, B. Verrucose setae; C. Long conidiophore; D, E. Short conidiophores; F–H. Conidia; I, J. Separating cells. Scale bars: A–C = 10 µm; D–J = 5 µm.

or slightly curved, simple or rarely branched in the apical region, septate, verrucose, dark-brown, lighter in the apical region, 170–432 × 5–9.4 µm, and short conidiophores single or in groups with long conidiophores, erect, straight or slightly curved, simple or branched, septate, smooth, pale brown, 8–20 × 4–6 µm. Conidiogenous cells terminal, cylindrical, 6–12 × 4–6 µm, polyblastic, integrated, sympodial, denticulate, pale brown. Separating cells ellipsoid to subglobose, 7–10 × 2.6–4 µm, smooth, subhyaline, with one denticle on each end. Conidia originated directly from conidiogenous cells in the long conidiophores and from separating cells in short conidiophores, turbinated, obovate to obpyriform, subhyaline, 0-septate, 18–24.6 × 4.6–9 µm, with a supraequatorial subhyaline transverse band.

Specimens examined — **BRAZIL. Bahia: Una**, on decaying leaves of *Myrcia splendens*, 22/IX/2011, M.V.O.dos Santos 1360 (CEPEC 2361); on decaying leaves of

Pera glabrata, 22/IX/2011, M.V.O.dos Santos 1361 (CEPEC 2362); on decaying leaves of *Inga thibaudiana*, 2/IV/2012, M.V.O.dos Santos 1362 (CEPEC 2363).

Geographical distribution — Japan (Shirouzu et al. 2010), Brazil (this paper).

Comments — *Beltraniella botryospora* was previously described as associated with living and dead leaves of *Quercus acuta* Thunb. and *Q. salicina* Blume in Japan (Shirouzu et al. 2010). In Brazil, *B. botryospora* is now being reported in association with the leaf litter of three trees belonging to different families, none closely related to *Fagaceae*, clearly indicating its adaptability to a wide range of substrates.

The specimen's characters are consonant with the original description (Shirouzu et al. 2010). However, in the original description, the setae were larger (140–550 µm), the conidiophores were shorter (12–30 µm), and the conidiogenous cells were larger (10–20 µm), while the separating cells (5 µm diam.) and conidia (7.5–10 µm diam.) were narrower. Young and immature structures were not considered for measuring the dimensions of the setae.

Beltraniella botryospora is similar to *B. fertilis* due to the presence of setae, two types of conidiophores (long setiform conidiophores and short non-setiform conidiophores) with fertile apices, and turbinate conidia (Heredia et al. 2002). However, the conidiophores of *B. fertilis* branch more than once at the apex, whereas branching is rare in *B. botryospora* (Shirouzu et al. 2010); also, *B. fertilis* has smaller setae and narrower conidia (Heredia et al. 2002). *Beltraniella botryospora* is also similar to *B. portoricensis*, which differs by producing only one type of conidiophore (short non-setiform conidiophores; Pirozynski & Patil 1970).

This is the first record of *Beltraniella botryospora* in the Americas.

Beltraniella portoricensis (F. Stevens) Piroz. & S.D. Patil, Can. J. Bot. 48: 575 (1970)

Description and illustration: Ellis (1971, as *Ellisiopsis gallesiae*), Gusmão & Grandi (1996).

Specimens examined — **BRAZIL. Bahia: Una**, on decaying leaves of *Inga thibaudiana*, 22/IX/2011, M.V.O.dos Santos 1363 (CEPEC 2364); on decaying leaves of *Pera glabrata*, 22/IX/2011, M.V.O.dos Santos 1364 (CEPEC 2365); on decaying leaves of *Myrcia splendens*, 22/IX/2011, M.V.O.dos Santos 1365 (CEPEC 2366).

Geographical distribution — Cosmopolitan.

Comments — No previous records were found of associations between *B. portoricensis* and the three plants studied, and this is the first record of this taxon for the county of Una. *Beltraniella portoricensis* was found only in the first sampling. This species is commonly found in the leaf litter of various plants occurring in tropical, subtropical, and temperate regions (Grandi & Gusmão 2002) and appears to be adaptable regarding substrate (Hyde et al. 2007).

Key to *Beltraniella* species cataloged in Brazil

1a. Setae and separating cells present 2
1b. Setae and separating cells absent ..... *B. japonica*
2a. Setiform conidiophores absent 3
2b. Setiform conidiophores present 4
3a. Setae smooth; separating cells clavate, often remaining attached on the conidial base ..... *B. amoena*
3b. Setae verrucose; separating cells oval to fusiform ..... *B. portoricensis*
4a. Conidiophore, simple or rarely branched at apex, with branching occurring once at most, up to 560 µm long; setae up to 550 µm long; separating cells subglobose to ovoid ..... *B. botryospora*
4b. Conidiophore, branched at the apex more than once, up to 328 µm long; setae up to 202 µm long; separating cells obovoid ..... *B. fertilis*

Acknowledgements

We thank the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) for the grant awarded to the first author and the Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) for grants awarded to the last two authors; the Reserva Biológica de Una, the Programa de Pós-Graduação em Biologia de Fungos (PPGBF) of the Universidade Federal de Pernambuco (UFPE) and José Lima da Paixão for assisting us in the sampling; and the Comissão Executiva do Plano da Lavoura Cacaueira (CEPLAC) for providing the laboratory to conduct the research. The authors also thank Drs. Álvaro Figueredo dos Santos and Nadja Santos Vitória for the pre-submission review for our manuscript.

Literature cited

Ellis MB. 1971. Dematiaceous hyphomycetes. Commonwealth Mycological Institute, Kew.

Grandi RAP, Gusmão LFP. 2002. Hyphomycetes decompositores do folhedo de *Tibouchina pulchra* Cogn. Revista Brasileira de Botânica 25: 79–87. http://dx.doi.org/10.1590/S0100-84042002000100010

Gusmão LFP, Grandi RAP. 1996. Espécies do grupo *Beltrania* (hyphomycetes) associadas a folhas de *Cedrela fissilis* Vell. (*Meliaceae*), em Maringá, PR, Brasil. Hoehnea 23: 91–102.

Gusmão LFP, Grandi RAP, Milanez AI. 2001. Hyphomycetes from leaf litter of *Miconia cabussu* in the Brazilian Atlantic rain forest. Mycotaxon 79: 201–213.

Heredia G, Arias RM, Reyes M, Castañeda-Ruiz R. 2002. New anamorph fungi with rhombic conidia from Mexican tropical forest litter. Fungal Diversity 11: 99–107.

Hyde KD, Bussaban B, Paulus B, Crous PW, Lee S, McKenzie EHC, Photita W, Lumyong S. 2007. Diversity of saprobic microfungi. Biodiversity and Conservation 16: 7–35. http://dx.doi.org/10.1007/s10531-006-9119-5

Kirk PM. 1981. New or interesting microfungi III. A preliminary account of microfungi colonizing *Laurus nobilis* leaf litter. Transactions of the British Mycological Society 77: 457–473. http://dx.doi.org/10.1016/S0007-1536(81)80093-9

Kirk PM, Cannon PF, Minter DW, Stalpers JA (eds). 2008. Dictionary of the Fungi, 10th edition. CAB International, Wallingford.

Magalhães DMA, Luz EDMN, Magalhães AF, Santos Filho LPdos, Loguercio LL, Bezerra JL. 2011. Riqueza de fungos anamorfos na serapilheira de *Manilkara maxima, Parinari alvimii* e *Harleyodendron unifoliolatum* na Mata Atlântica do Sul da Bahia. Acta Botânica Brasílica 25: 899–907. http://dx.doi.org/10.1590/S0102-33062011000400017

Marques MFO, Barbosa FR, Gusmão LFP, Castañeda-Ruiz RF, Maia LC. 2007. Conidial fungi from the semi-arid Caatinga biome of Brazil. *Cubasina microspora* sp. nov., a note on *C. albofusca*, and some new records for South America. Mycotaxon 102: 17–23.

Pirozynski KA, Patil SD. 1970. Some setose hyphomycetes of leaf litter in south India. Canadian Journal of Botany 48: 567–581.

Polishook JD, Bills GF, Lodge DJ. 1996. Microfungi from decaying leaves of two rain forest trees in Puerto Rico. Journal of Industrial Microbiology 17: 284–294. http://dx.doi.org/10.1007/BF01574703

Priya SW, Nagaveni HC, Kunwar IK, Manoharachary IK. 2011. A new pathogenic species of *Beltraniella* from India. Journal of Mycology and Plant Pathology 41: 20–23.

Seifert K, Morgan-Jones G, Gams W, Kendrick B. 2011. The genera of hyphomycetes. CBS Biodiversity Series 9. 997 p.

Shirouzu T, Hirose D, Fukasawa Y, Tokumasu S. 2009. Fungal succession associated with the decay of leaves of an evergreen oak, *Quercus myrsinaefolia*. Fungal Diversity 34: 87–109.

Shirouzu T, Hirose D, Tokumasu S, To-Anun C, Maekawa N. 2010. Host affinity and phylogenetic position of a new anamorphic fungus *Beltraniella botryospora* from living and fallen leaves of evergreen oaks. Fungal Diversity 43: 85–92. http://dx.doi.org/10.1007/s13225-010-0037-1

Silva P, Grandi RAP. 2011. A new species of *Thozetella* (anamorphic fungi) from Brazil. Cryptogamie, Mycologie 32: 359–363. http://dx.doi.org/10.7872/crym.v32.iss4.2011.359

Subramanian CV. 1952. Fungi imperfecti from Madras—III. *Beltraniella* gen. nov. Proceedings of the Indian Academy of Sciences, Section B, 36: 223–228.

ISSN (print) 0093-4666 © 2014. Mycotaxon, Ltd. ISSN (online) 2154-8889

MYCOTAXON

http://dx.doi.org/10.5248/129.7

Volume 129(1), pp. 7–20 *July–September 2014*

Neofomitella polyzonata gen. et sp. nov., and *N. fumosipora* and *N. rhodophaea* transferred from *Fomitella*

Hai-Jiao Li[1,2], Xing-Chun Li[1], Josef Vlasák[3], & Yu-Cheng Dai[1]*

[1] *Institute of Microbiology, Beijing Forestry University, Beijing, 100083, China*

[2] *National Institute of Occupational Health and Poison Control, Chinese Center for Disease Control and Prevention, Beijing, 100050, China*

[3] *Biology Centre of the Academy of Sciences of the Czech Republic, Branišovská 31, CZ-370 05 České Budějovice, Czech Republic*

*Correspondence to: *daiyucheng2013@gmail.com*

Abstract — Phylogenetic analysis based on ITS, nLSU, and RPB2 sequences revealed that *Fomitella* in the current sense belonged to two distantly related subclades in the core polyporoid clade. *Fomitella* in a narrow sense is proposed for the type species, *F. supina*, and *Neofomitella* gen. nov. is proposed for *Fomitella fumosipora* and *F. rhodophaea* and a new species *N. polyzonata*. *Neofomitella* differs from *Fomitella* by its distinctly crusted basidiocarps with the cuticle developing from base to margin. Illustrated descriptions of the new genus and species are provided. The main morphological differences between *Fomitella*, *Neofomitella*, and related genera are discussed, and an identification key to *Neofomitella* is also provided.

Key words — phylogeny, *Polyporaceae*, *Polyporales*, *Basidiomycota*, taxonomy

Introduction

Fomitella Murrill, typified by *F. supina* (Sw.) Murrill, was erected as a monotypic genus by Murrill (1905). Subsequently, *F. fumosoavellanea* (Romell) Murrill [*Trichaptum fumosoavellaneum* (Romell) Rajchenb. & Bianchin.], *F. fumosipora*, *F. rhodophaea*, and *F. malaysiana* (Corner) T. Hatt. & Sotome were transferred to the genus (Murrill 1908; Hattori 2005; Hattori & Sotome 2013). Hattori (2005) emended the generic concept of *Fomitella* as follows: basidiocarps annual to perennial, effused-reflexed to distinctly pileate, pileal surface glabrous to minutely tomentose, context firm-fibrous to corky, light orange to pale brown, with a dark agglutinated crust; hyphal system trimitic with clamped generative hyphae, skeletal and binding hyphae well differentiated, negative in Melzer's reagent; cystidia absent; basidiospores

ellipsoid to cylindrical, colorless, thin-walled, smooth, and negative in Melzer's reagent; causing a white rot.

On-going studies on the diversity of wood-rotting fungi in eastern China have produced several new species (Dai & Cui 2005; Cui & Dai 2008a,b; Cui et al. 2007, 2008; He & Dai 2012; Wang et al. 2009, 2011; Cao et al. 2012), and additional study on the specimens from this area recently revealed an undescribed species. Phylogenetic analysis clustered our undescribed species with *Fomitella fumosipora* and *F. rhodophaea* as a clade separated from *F. supina*. Therefore, we propose a new genus *Neofomitella* to accommodate the new species and two new combinations from *Fomitella*.

Materials & methods

Morphological studies

The studied specimens were deposited at the herbaria of the Institute of Microbiology, Beijing Forestry University (BJFC) and Institute of Applied Ecology, Chinese Academy of Sciences (IFP). The microscopic procedure follows Cui & Zhao (2012). To present the basidiospore size variation, 5% of measurements were excluded from each end of the range and were given in parentheses. In the text the following abbreviations were used: IKI = Melzer's reagent, IKI– = both inamyloid and non-dextrinoid, KOH = 5% potassium hydroxide, CB = Cotton Blue, CB– = acyanophilous, L = mean spore length (arithmetic average of all spores), W = mean spore width (arithmetic average of all spores), Q = variation in the L/W ratios between the specimens studied, n = number of spores measured from given number of specimens. Special colors follow Petersen (1996).

Molecular study and phylogenetic analysis

Dried specimens were used for molecular study. CTAB rapid Plant genome extraction kit-DN14 (Aidlab Biotechnologies Co. Ltd, Beijing) was used to extract DNA, according to the manufacturer's instructions with some modifications. ITS region was amplified with primer pair ITS5 and ITS4 (White et al. 1990), nLSU region with primer pair LR0R and LR7 (http://www.biology.duke.edu/fungi/mycolab/primers.htm), and RPB2 with primer pair fRPB2-f5F and bRPB2-7.1R (Liu et al. 1999; Matheny 2005). The PCR procedures were: (1) for ITS — initial denaturation at 95°C for 3 min, followed by 35 cycles at 94°C for 40 s, 54°C for 45 s and 72°C for 1 min, and a final extension of 72°C for 10 min; (2) for nLSU — initial denaturation at 94°C for 1 min, followed by 35 cycles at 94°C for 30 s, 50°C for 1 min and 72°C for 1.5 min, and a final extension of 72°C for 10 min; and (3) for RPB2 — initial denaturation at 94°C for 2 min, followed by 10 cycles at 94°C for 40 s, 60°C for 40 s and 72°C for 2 min, then followed by 37 cycles at 94°C for 45 s, 55°C for 1.5 min and 72°C for 2 min, and a final extension of 72°C for 10 min. The PCR products were directly sequenced in Beijing Genomics Institute, China, with the same primers.

Other reference sequences for our phylogenetic analysis were selected from Binder et al. (2005), Justo & Hibbett (2011), Miettinen & Rajchenberg (2012), and BLAST searches in GenBank (Table 1). These sequences were sampled from *Polyporales* with

TABLE 1. Polyporalean and outgroup taxa used in phylogenetic analysis.
(Sequences generated in this study are in bold.)

SPECIES	SAMPLE NUMBER	ITS	nLSU	RPB2
Abundisporus pubertatis	Dai 11927	**KC867398**	**KC867494**	**KF274654**
Abundisporus violaceus	MUCL38617	FJ411100	FJ393867	—
Climacodon septentrionalis	AFTOL-ID 767	AY854082	AY684165	AY780941
Coriolopsis aspera	Cui 6702	**KC867353**	**KC867476**	**KF274658**
	Cui 6725	**KC867356**	**KC867477**	**KF274659**
Coriolopsis brunneoleuca	Dai 12087	**KC867416**	**KC867435**	**KF274656**
	Dai 12180	**KC867414**	**KC867432**	**KF274655**
Coriolopsis byrsina	FP-105050-Sp	JN165001	JN164788	JN164871
Coriolopsis cf. *caperata*	CR22	JN164999	JN164789	JN164870
	Ryvarden 45481	**KC867399**	**KC867428**	**KF274657**
Coriolopsis retropicta	Dai 9870	**KC867404**	**KC867443**	**KF274653**
Coriolopsis rigida	BJFC12680	**KC867381**	**KC867454**	**KF274664**
Coriolopsis sanguinaria	Cui 5444	**KC867387**	**KC867463**	—
	Dai 9314	**KC867390**	**KC867467**	—
Coriolopsis sp.	BRFM1125	JX082370	—	—
	BRFM1126	JX082371	—	—
Coriolopsis strumosa	Dai 10642	**JX559278**	**JX559303**	**JX559312**
	Dai 10657	**KC867371**	**KC867491**	**KF274650**
Daedaleopsis confragosa	Cui 9732	**JX569731**	**JX569748**	**KF274647**
Daedaleopsis sinensis	Dai 11431	**JX569732**	**JX569749**	**KF274648**
Datronia mollis	RLG6304sp	JN165002	JN164791	JN164872
Datronia scutellata	RLG9584T	JN165004	JN164792	JN164873
Dentocorticium sulphurellum	T609	JN165015	JN164815	JN164875
Donkioporia expansa	P188	HM536087	HM536052	HM536102
Earliella scabrosa	PR1209	JN165009	JN164793	JN164866
Fomes fomentarius	Cui 8020	JX290073	JX290070	—
Fomitella supina	JV0610	**KF274645**	**KF274646**	—
	Nunez 1183	**KF274644**	—	—
	Ryvarden 39027	**KF274643**	—	—
Fomitopsis pinicola	AFTOL-ID 770	AY854083	AY684164	AY786056
Funalia gallica	BJFC12697	**KC867379**	**KC867453**	—
	RLG-7630-sp	JN165013	JN164814	JN164869
Funalia trogii	RLG-4286-Sp	JN164993	JN164808	JN164867
Ganoderma tsugae	AFTOL-ID 771	DQ206985	AY684163	DQ408116
Grifola sordulenta	AFTOL-ID 562	AY854085	AY645050	AY786058
Hexagonia apiaria	Cui 6447	KC867362	KC867481	KF274660
Hexagonia glabra	Cui 8468	**JX559277**	**JX559302**	**JX559311**
	Dai 10991	**JX569733**	**JX569750**	**KF274649**
Lignosus rhinocerotis	PEN94	JQ409359	AB368074	AB368132
Lopharia cinerascens	FP-105043-sp	JN165019	JN164813	JN164874
Megasporia major	Cui 10253	JQ314366	JQ780437	JX559314
Megasporoporiella subcavernulosa	Cui 9252	JQ780378	JQ78041	JX559315
Microporus affinis	Cui 7714	**JX569739**	**JX569746**	**KF274661**
Microporus flabelliformis	Dai 11574	**JX569740**	**JX569747**	**KF274662**
Microporus xanthopus	Cui 8284	JX290074	JX290071	JX559313
Neofomitella fumosipora	Cui 8816	**JX569734**	**JX569741**	—
	Dai 10777	**JX569735**	**JX569742**	—
Neofomitella polyzonata	Dai 10419	**JX569738**	**JX569745**	**KF274663**
	Dai 10420	**JX569736**	**JX569743**	—
	Dai 11360	**JX569737**	**JX569744**	—
Neofomitella rhodophaea	TFRI 414	EU232216	EU232300	—
Perenniporia corticola	Cui 1465	JN048759	JN048779	**KF274651**
Perenniporia tenuis	Wei 2783	JQ001858	JQ001848	**KF274652**
Phlebia radiata	FPL6140	AY854087	AF287885	AY218502
Polyporus grammocephalus	WD2343	AB587626	AB368089	AB368146
Polyporus varius	WD2347	AB587636	AB368111	AB368168
Pseudofavolus cucullatus	WD2157	AB587637	AB368114	AB368170
Trametes betulina	HHB-9942-sp	JN164983	JN164794	JN164860
Trametes elegans	FP-105679-sp	JN164944	JN164799	JN164861
Trametes polyzona	BKW-004	JN164978	JN164790	JN164856
Trametes sanguinea	PR-SC-95	JN164982	JN164795	JN164858
Trametes suaveolens	FP-102529-sp	JN164966	JN164807	JN164853
Trametes versicolor	FP-135156-sp	JN164919	JN164809	JN164850
Trametopsis cervina	TJV-93-216-sp	JN165020	JN164796	JN164877
Boletopsis leucomelaena	AFTOL-ID 1527	DQ484064	DQ154112	GU187820
Hydnellum geogenium	AFTOL-ID 680	DQ218304	AY631900	DQ408133

the aim of providing representatives for all major clades within the order, concluded by Larsson et al. (2004), Binder et al. (2005) and Larsson (2007). *Boletopsis leucomelaena* (Pers.) Fayod and *Hydnellum geogenium* (Fr.) Banker were selected as outgroup (Justo & Hibbett 2011). Phylogenetic analysis for each single gene was carried out, and we got similar topologies to combined dataset in both MP and Bayes analyses. So in the paper we showed the result from the combined dataset that received higher support values. This combined dataset was aligned using Clustalx 1.83 (Chenna et al. 2003) and manually edited as necessary. Sequence alignment was deposited at TreeBase (submission ID 14418).

Maximum parsimony (MP) analysis was performed using PAUP* 4.0b10 (Swofford 2002) with gaps treated as missing data. Trees were generated using 100 replicates of random stepwise addition of sequence and tree-bisection reconnection (TBR) branch-swapping algorithm. All characters were given equal weight. Branch support for all parsimony analysis was estimated by performing 1 000 bootstrap (BP) replicates (Felsenstein 1985) with a heuristic search of 10 random-addition replicates for each bootstrap replicate.

The best-fit model of nucleotide substitution was selected by hierarchical likelihood ratio tests (hLRT, Huelsenbeck & Crandall 1997; Posada & Crandall 2001) implemented in the MrModelTest 2.2 (Posada & Crandall 1998; Nylander 2004). Following this model, MrBayes 3.1.2 (Ronquist & Huelsenbeck 2003) was used for Bayesian inference (BI). Eight Markov chains were run from random starting tree for 3,000,000 generations and sampled every 100 generations. The first one-fourth of the trees, which represented the burn-in phase of the analysis, were discarded, while the last three-fourths were used for calculating Bayesian posterior probabilities (BPPs) in the consensus tree. Confident branch support is defined as BPPs not less than 0.95 and bootstrap values above 50%.

Results

Molecular phylogeny

The combined dataset included 66 samples with 66 ITS, 62 nLSU, and 51 RPB2 sequences, representing 50 species and two collections not identified to species level from the main clades of *Polyporales*, as well as two species from *Thelephorales* as outgroup. MP analysis yielded eight equally parsimonious trees (tree length = 9 153, CI = 0.283, RI = 0.520, RC = 0.147, HI = 0.717). The best model for the alignment was estimated as "GTR+I+G". BI resulted in an average standard deviation of split frequencies 0.005127. The topologies from MP and BI were similar, and only the BI tree was presented along with the BP from MP analysis (Fig. 1). In the phylogenetic analysis, sampled species of *Fomitella* formed two subclades within the core polyporoid clade. One subclade comprised *Fomitella supina*, the generic type, and two unidentified collections (labelled as *Coriolopsis*) from French Guiana (BPP = 1.00, MP = 100%; Fig. 1), while the second subclade included *F. fumosipora*, *F. rhodophaea* and our undescribed species (BPP = 1.00, MP = 96%; Fig. 1). Taken the morphology into consideration together, we propose a new genus, *Neofomitella*, to accommodate

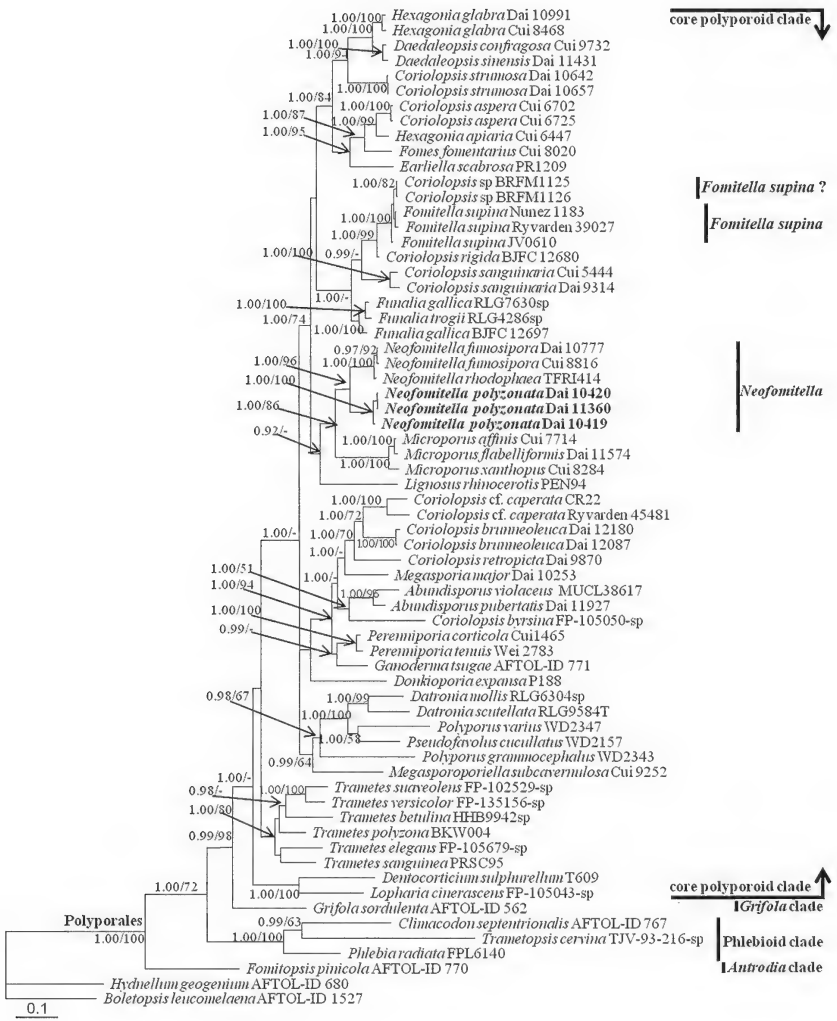


FIG. 1. Phylogram of polyporalean taxa obtained from Bayesian inference of the combined dataset of ITS, nLSU, and RPB2. Bayesian posterior probabilities more than 0.95 and bootstrap values above 50% are indicated above or below the branches. The scale bar represents estimated number of changes per site.

the second subclade; we transfer the two *Fomitella* species to the new genus and describe our new species as *N. polyzonata*. Phylogenetically, the *Neofomitella* subclade clusters with a *Microporus* subclade (FIG. 1).

Taxonomy

Neofomitella Y.C. Dai, Hai J. Li & Vlasák, **gen. nov.**

MycoBank MB 804799

Differs from *Fomitella* by its distinctly crusted basidiocarps with the cuticle developing from base to margin and from *Microporus* by its buff, yellowish brown, brown to pale grey context.

Type species — *Polyporus rhodophaeus* Lév.

Etymology — *Neofomitella* (Lat.): referring to the morphological similarity to *Fomitella*.

Basidiocarps annual or perennial, pileate, sessile or effused-reflexed. Pileal surface yellowish-brown, brown, orange-brown, reddish-brown, fuscous to almost black, usually concentrically zonate or sulcate, glabrous to velutinate. Context buff, yellowish brown, brown to pale grey, corky to hard corky, with a dark agglutinated crust developing from base to margin. Pore surface usually white, cream to pale buff when fresh, pale brown to yellowish-brown when dry. Hyphal system trimitic with clamped generative hyphae, skeletal and binding hyphae well differentiated, negative in Melzer's reagent. Cystidia absent. Basidiospores oblong ellipsoid to cylindrical, hyaline, thin-walled, smooth, IKI–, CB–, tissue turn into black in KOH. Growing usually on angiosperm wood and causing a white rot.

Neofomitella fumosipora (Corner) Y.C. Dai, Hai J. Li & Vlasák, **comb. nov.** Fig. 2a

MycoBank MB 804803

≡*Trametes fumosipora* Corner, Beih. Nova Hedwigia 97: 106 (1989).

≡*Fomitella fumosipora* (Corner) T. Hatt., Mycoscience 46: 309 (2005).

Specimens examined — **China**. **Guangdong Province**, Shixing County, Chebaling Nature Reserve, fallen angiosperm trunk, 25 Jun 2010, B.K. Cui 8816 (BJFC 7756); 23 Nov 2010, B.K. Cui 8715, 8717 (BJFC 7657, 7659); **Hainan Province**, Changjiang County, Bawangling Nature Reserve, fallen angiosperm trunk, 8 May 2009, Y.C. Dai 10777 (BJFC 5021). **Malaysia**. **Negeri Sembilam**, Pasoh For. Res., 5 Dec 1998, T. Hattori (BJFC: ex. TFM ex. F. 19017); **Penang**, Penang Hill, alt. 500–750 m, 13 Dec 2002, T. Hattori (BJFC: ex. TFM ex. F. 20477).

Neofomitella polyzonata Y.C. Dai, Hai J. Li & Vlasák, **sp. nov.** Figs 2b,c, 3

MycoBank MB 804804

Differs from *Neofomitella fumosipora* and *N. rhodophaea* by its distinctly velutinate pileal surface and larger pores.

Type — China, Jiangxi Province, Fenyi County, Dagang Mountain, on fallen trunk of *Cyclobalanopsis blakei* (Skan) Schottky (*Fagaceae*), 18 Sep 2008, Y.C. Dai 10419 (**holotype**, BJFC 4668).

Etymology — *polyzonata* (Lat.): referring to the multiple zones on the pileal surface.

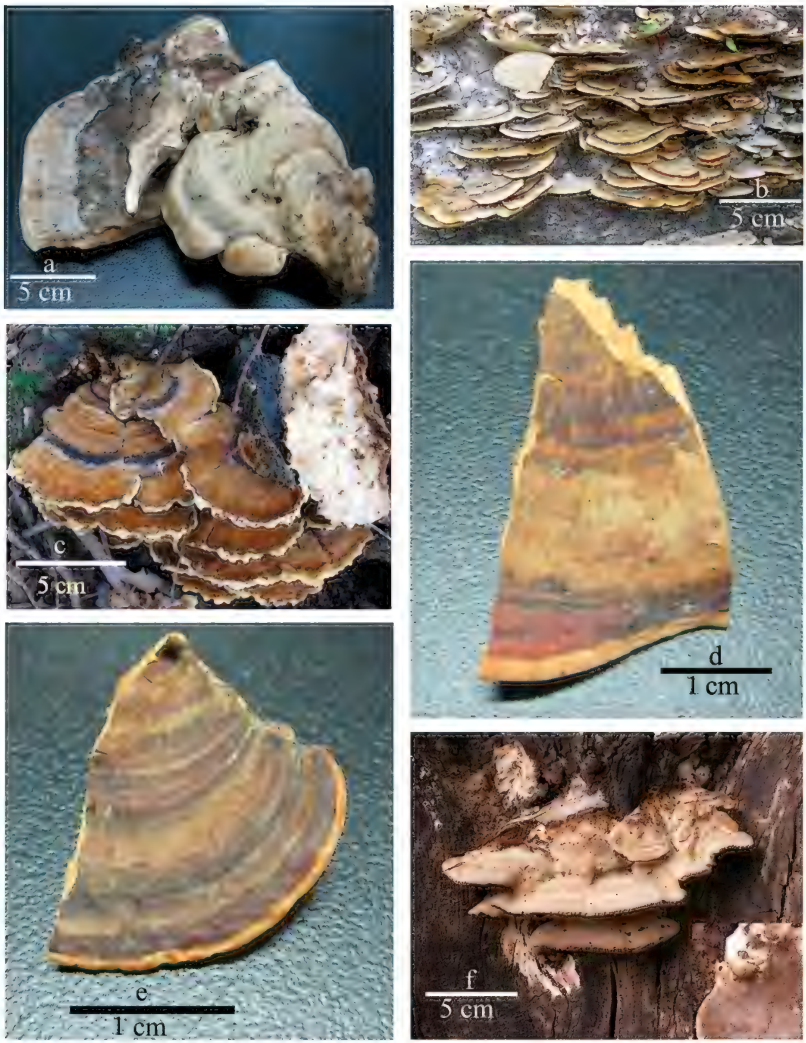


Fig. 2. *Neofomitella* and *Fomitella* basidiocarps. a: *N. fumosipora*. b, c: *N. polyzonata*. d, e: *N. rhodophaea*. f: *F. supina*.

Fruitbody — Basidiocarps annual, pileate, sessile, usually imbricate, without odor or taste when fresh, hard corky to woody hard and light in weight upon drying. Pilei applanate, semicircular to dimidiate, up to 6 cm long, 10 cm wide and 6 mm thick at base. Pileal surface buff-yellow, curry-yellow, cinnamon, orange-brown to reddish brown, with one or more vinaceous brown, dark blue

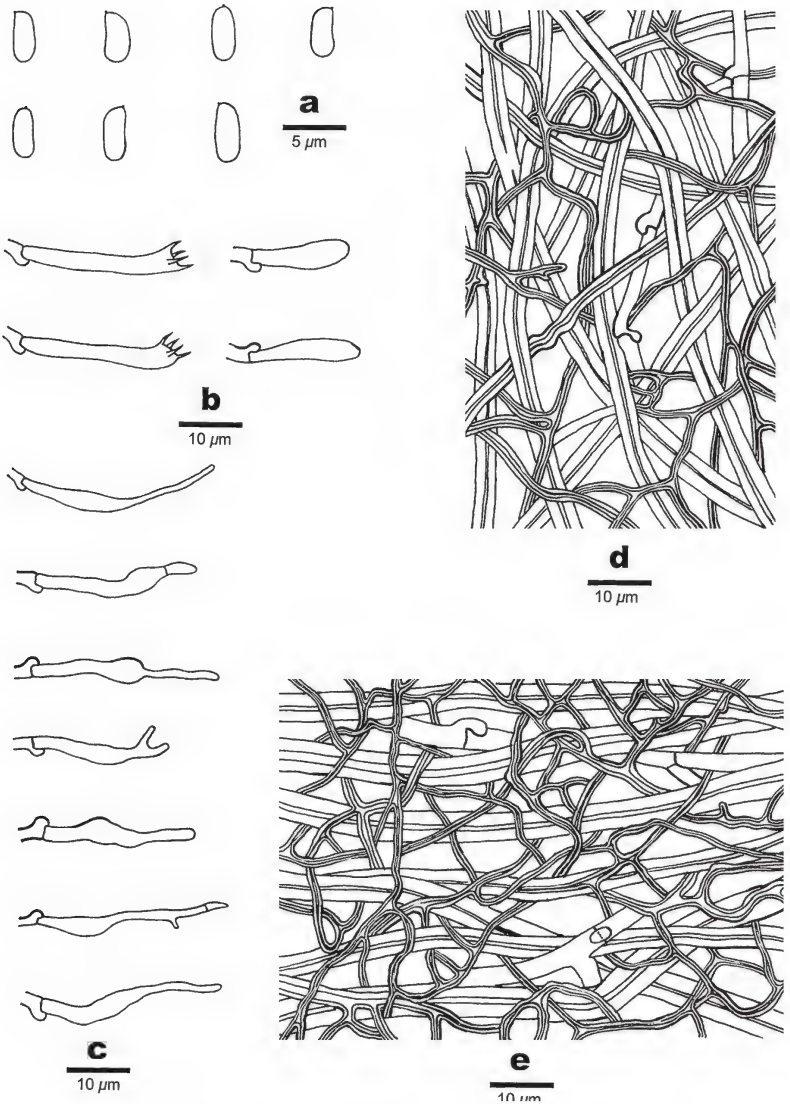


Fig. 3. *Neofomitella polyzonata* (drawn from the holotype).
a: Basidiospores. b: Basidia and basidioles. c: Cystidioles.
d: Hyphae from trama. e: Hyphae from context.

to almost black zones, finely velutinate, concentrically zonate; white to cream outgrowth occasionally spreading from the base with age. Margin cream, buff to buff-yellow, usually acute or slightly wavy. Pore surface cream to buff when

fresh, buff to yellowish brown when dry or bruised; sterile margin indistinct, white to cream, up to 0.5 mm wide; pores round, 3–4 per mm; dissepiments thin, entire. Context buff to yellowish brown, hard corky, azonate, up to 3 mm thick, a more or less dark agglutinated crust present as black zones in context towards upper surface. Tube layer concolorous with pore surface, up to 3 mm long.

Hyphal structure — Hyphal system trimitic; generative hyphae bearing clamp connections; skeletal and binding hyphae IKI–, CB–; tissue turning into black in KOH.

Context — Generative hyphae in context infrequent, colorless, thin-walled, moderately branched, 1.5–3 μm in diam; skeletal hyphae in context dominant, colorless to pale yellowish brown, thick-walled with a narrow lumen to subsolid, occasionally branched, straight, more or less regularly arranged, 3–5 μm in diam; binding hyphae in context abundant, colorless to pale yellowish brown, thick-walled with a narrow lumen to subsolid, flexuous, frequently branched, interwoven, 1–2.5 μm in diam.

Tubes — Generative hyphae infrequent, colorless, thin-walled, moderately branched, 1.2–2 μm in diam; skeletal hyphae in trama dominant, colorless to pale yellowish brown, thick-walled, occasionally branched, more or less straight, interwoven, 2.4–4 μm in diam; binding hyphae in trama colorless to pale yellowish brown, thick-walled with a narrow lumen to subsolid, flexuous, frequently branched, interwoven, 1–2 μm in diam. Cystidia absent, while cystidioles present, fusoid to tubular, sometimes tips of cystidioles branched and septate, colorless, thin-walled, 22–34 × 2.5–4.5 μm; basidia clavate, bearing four sterigmata and a basal clamp connection, 18–24 × 3.5–5 μm; basidioles in shape similar to basidia, but distinctly smaller.

Spores — Basidiospores cylindrical, colorless, thin-walled, smooth, IKI–, CB–, (3.8–)3.9–5 × (1.8–)1.9–2.1(–2.5) μm, L = 4.34 μm, W = 2.04 μm, Q = 2.13 (n = 9/1).

Type of rot —White rot.

Additional specimens examined — **CHINA. Fujian Province**, Wuyi Mountains, Longfenggu Forest Park, on fallen angiosperm trunk, 27 Aug 2006, B.K. Cui 4124 (BJFC 485, IFP 1196); Wuyishan Nature Reserve, Taoyuanyu, on fallen angiosperm trunk, 24 Aug 2006, Y.C. Dai 7376 (IFP 11887); **Hunan Province**, Shimen County, Hupingshan Nature Reserve, on fallen angiosperm trunk, 16 Sep 2009, Y.C. Dai 11360 (BJFC 7283); **Jiangxi Province**, Fenyi County, Dagang Mountain, on fallen trunk of *Cyclobalanopsis blakei*, 18 Sep 2008, Y.C. Dai 10420 (BJFC 4669).

Neofomitella rhodophaea (Lév.) Y.C. Dai, Hai J. Li & Vlasák, **comb. nov.** Figs 2d,e

MycoBank MB 804800

≡ *Polyporus rhodophaeus* Lév., Ann. Sci. Nat., Bot., 3e Sér., 2: 190 (1844).

≡ *Fomitella rhodophaea* (Lév.) T. Hatt., Mycoscience 46: 305 (2005).

Specimens examined — **JAPAN**. **Kagoshima Pref.**, Kimotsuki-Gun, Uchindura, Oct 1962, K. Aoshima (BJFC: ex. O 10866); **Tokyo**, Meguro, 15 Oct 1943 (BJFC: ex. TFM F-10861); **MALAYSIA**. **Negeri Sembilan**, Pasoh, Sembilan For. Nat. Res., 25 Dec 1997, T. Hattori (BJFC: ex. TFM 18313).

Other Specimens examined — ***Fomitella supina*** (Fig. 2f): **BRAZIL**. **Alagoas State**, Pilar Municipality, Reserva Particular do patrimonio Natural (PPPN) São Pedro, on dead hardwood, Oct 2000, B. Tatiana, N. Gibertoni 357 (BJFC: ex. O 10849). **COLOMBIA**. **Dept. de Antioquia**, Municipio Caldas finca, alt. 1700 m, 25 Jun 1978, L. Ryvarden 16 590 (BJFC: ex. O 10769). **GUATEMALA**. Lago Atitlán, on hard wood, J. Vlasák JV0610. **PANAMA**. Ensenada de Santa Cruz. Parque Nac. de Coiba, 17 Nov 1996, M. Nunez 1183 (BJFC: ex. O 10770). **PUERTO RICO**. Toro Negro, Commonwealth For., on deciduous wood, 24 Jun 1996, L. Ryvarden 39 027 (BJFC: ex. O 10772).

Discussion

The new genus *Neofomitella* is composed of two distinct lineages, one comprising *Neofomitella rhodophaea* and *N. fumosipora* and the other *N. polyzonata*, newly described from China. The main morphological characters of *Fomitella supina* and the three *Neofomitella* species are presented in Table 2. Like *Neofomitella polyzonata*, *N. fumosipora* has more or less brown basidiocarps; however, it has a glabrous pileal surface and smaller pores (7–9 per mm; Hattori 2005). All three *Neofomitella* species show distinctly crusted basidiocarps with a cuticle that develops from base to margin (Fig. 2a-e). In *F. supina* the cuticle also develops from the base but does not usually extend to the very margin (Fig. 2f).

Neofomitella is phylogenetically close to several *Microporus* species (Fig. 1). Although *Microporus* also has a trimitic hyphal system, colorless, thin-walled, smooth, non-dextrinoid, inamyloid basidiospores, its species usually produce stipitate basidiocarps with a white to cream context (Gilbertson & Ryvarden 1986; Núñez & Ryvarden 2001). Its type species, *M. perula* P. Beauv. [= *M. xanthopus* (Fr.) Kuntze], also has an encrusted pileal surface, but its centrally or laterally stipitate and usually infundibuliform basidiocarps, distinctly small pores, and white context (Núñez & Ryvarden 2001) clearly differentiate it from *Neofomitella* species.

Morphologically, *Coriolopsis* shares many features with *Fomitella* and *Neofomitella*, such as a more or less brown context, trimitic hyphal system with clamped generative hyphae, colorless basidiospores, and causing a white rot (Gilbertson & Ryvarden 1986; Núñez & Ryvarden 2001; Hattori 2005). However, phylogenetic analysis shows *Coriolopsis* as polyphyletic with its type species, *Polyporus occidentalis* Klotzsch [≡ *Coriolopsis occidentalis* (Klotzsch) Murrill; = *Trametes polyzona* (Pers.) Justo], clustering within the *Trametes* clade and distinctly separated from *Fomitella* and *Neofomitella* (Fig. 1).

Table 2. Main morphological characters of *Fomitella* and *Neofomitella* species.

Species	Basidiocarps[1]	Pores (/mm)	Basidiospores[2] (µm)	Cystidioles[3]	Distribution[4]
F. supina	A/P, V then G	5–7	6.5–9 × 2.4–3.5, C	–	T/S, America, Africa
N. fumosipora	A/P, G	6–10	3–4 × 1.7–2.2, C/OE	+	T/S, Asia
N. polyzonata	A, V	3–4	3.9–5 × 1.9–2.1, C	+	S/WT, China
N. rhodophaea	A/P, G	7–8	3.5–4.5 × 2.5–3, OE	–	T, also S/WT, Asia, Africa

[1] A = annual, P = perennial, G = glabrous, V = velutinate; [2] C = cylindrical, OE = oblong-ellipsoid; [3] + = presence, – = absence; [4] T = tropics, S = subtropics, WT = warm-temperate.

The transfer of this taxon to *Trametes* was suggested by Corner (1989) and validated by Justo & Hibbett (2011). *Coriolopsis strumosa* (Fr.) Ryvarden usually has encrusted basidiocarps that are similar to *Neofomitella*, but *C. strumosa* has a soft corky, olivaceous-brown, umber or hazel-brown context and distinctly larger basidiospores (7–10 × 3–4 µm; Núñez & Ryvarden 2001; Li 2013).

Funalia species cluster in the same clade with *Fomitella*. Both genera share trimitic hyphal system and more or less similar basidiospores, but *Funalia* species usually produce a strongly tomentose to hispid pileal surface, a white, cream to straw colored context, and cyanophilous skeletal hyphae (Niemelä et al. 1992; Dai 1996).

Hexagonia has pileate brown basidiocarps, tissues that darken in KOH, a trimitic hyphal system, and colorless thin-walled basidiospores similar to those in *Fomitella* and *Neofomitella* (Núñez & Ryvarden 2001). However, *Hexagonia* species usually have larger hexagonal pores and distinctly larger basidiospores (usually longer than 10 µm; Gilbertson & Ryvarden 1986; Núñez & Ryvarden 2001).

No sequences of *Fomitella malaysiana* are available at present. The distinctly encrusted pileal surface (Hattori & Sotome 2013) indicates that this species may belong to *Neofomitella*. Further molecular studies are needed to resolve its taxonomic and phylogenetic position.

Key to species of *Neofomitella*

1. Pores 3–4 per mm ..... *N. polyzonata*
1. Pores 6–10 per mm 2
2. Basidiospores 1.7–2.2 µm wide ..... *N. fumosipora*
2. Basidiospores 2.5–3 µm wide ..... *N. rhodophaea*

Acknowledgments

We express our gratitude to Drs. Michal Tomšovský (Mendel University in Brno, Czech Republic) and Li-Wei Zhou (IFP, China) who reviewed the manuscript. Great thanks to Drs. Hai-Sheng Yuan (IFP, China) and Shuang-Hui He (BJFU, China) for help in field collections. Special thanks are due to Dr. Tsutomu Hattori (FFPRI, Japan), Prof. Leif Ryvarden (O, Norway) and Dr. Tatiana B. Gibertoni (URM, Brazil) for loan of specimens. The research is supported by the National Natural Science Foundation of China (Project No. 31093440), the Program for New Century Excellent Talents in University (NCET-11-0585), and the institutional support RVO: 60077344 of the Czech Academy of Sciences to J. Vlasák.

Literature cited

Binder M, Hibbett DS, Larsson KH, Larsson E, Langer E, Langer G. 2005. The phylogenetic distribution of resupinate forms across the major clades of mushroom-forming fungi (*Homobasidiomycetes*). Systematics and Biodiversity 3: 113–157. http://dx.doi.org/10.1017/S1477200005001623

Cao Y, Wu SH, Dai YC. 2012. Species clarification of the prize medicinal *Ganoderma* mushroom "Lingzhi". Fungal Diversity 56: 49–62. http://dx.doi.org/10.1007/s13225-012-0178-5

Chenna R, Sugawara H, Koike T, Lopez R, Gibson TJ, Higgins DG, Thompson JD. 2003. Multiple sequence alignment with the Clustal series of programs. Nucleic Acids Research 31: 3497–3500. http://dx.doi.org/10.1093/nar/gkg500

Corner, EJH. 1989. Ad Polyporaceas 6. The genus *Trametes*. Beihefte zur Nova Hedwigia 97: 1–197 .

Cui BK, Dai YC. 2008a. *Skeletocutis luteolus* sp. nov. from southern and eastern China. Mycotaxon 104: 97–101.

Cui BK, Dai YC. 2008b. Wood-rotting fungi in eastern China 2. A new species of *Fomitiporia* (*Basidiomycota*) from Wanmulin Nature Reserve, Fujian Province. Mycotaxon 105: 343–348.

Cui BK, Zhao CL. 2012. Morphological and molecular evidence for a new species of *Perenniporia* (*Basidiomycota*) from Tibet, southwestern China. Mycoscience 53: 365–372. http://dx.doi.org/10.1007/s10267-011-0180-x

Cui BK, Dai YC, Decock C. 2007. A new species of *Perenniporia* (*Basidiomycota*, *Aphyllophorales*) from eastern China. Mycotaxon 99: 175–180.

Cui BK, Yuan HS, Dai YC. 2008. Wood-rotting fungi in eastern China 1. Polypores from Wuyi Mountains, Fujian Province. Sydowia 60: 25–40.

Dai YC. 1996. Changbai wood-rotting fungi 7. A check list of the polypores. Fungal Science 11: 79–105.

Dai YC, Cui BK. 2005. Two new species of the *Hymenochaetaceae* from eastern China. Mycotaxon 94: 341–347.

Felsenstein J. 1985. Confidence intervals on phylogenies: an approach using the bootstrap. Evolution 39: 783–791.

Gilbertson RL, Ryvarden L. 1986. North American polypores 1. *Abortiporus–Lindtneria*. Fungiflora, Oslo. 433 p.

Hattori T. 2005. Type studies of the polypores described by E.J.H. Corner from Asia and West Pacific Areas 7. Species described in *Trametes* (1). Mycoscience 46: 303–312. http://dx.doi.org/10.1007/s10267-005-0250-z

Hattori T, Sotome K. 2013. Type studies of the polypores described by E.J.H. Corner from Asia and West Pacific areas VIII. Species described in *Trametes* (2). Mycoscience 54: 297–308. http://dx.doi.org/ 10.1016/j.myc.2012.10.008

He SH, Dai YC. 2012. Taxonomy and phylogeny of *Hymenochaete* and allied genera of *Hymenochaetaceae* (*Basidiomycota*) in China. Fungal Diversity 56:77–93. http://dx.doi.org/10.1007/s13225-012-0174-9

Huelsenbeck JP, Crandall KA. 1997. Phylogeny estimation and hypothesis testing using maximum likelihood. Annual Review of Ecology, Evolution, and Systematics 28: 437–466. http://dx.doi.org/10.1146/annurev.ecolsys.28.1.437

Justo A, Hibbett DS. 2011. Phylogenetic classification of *Trametes* (*Basidiomycota*, *Polyporales*) based on a five-marker dataset. Taxon 60: 1567–1583.

Larsson KH. 2007. Re-thinking the classification of corticioid fungi. Mycological Research 111: 1040–1063. http://dx.doi.org/10.1016/j.mycres.2007.08.001

Larsson KH, Larsson E, Köljalg U. 2004. High phylogenetic diversity among corticioid homobasidiomycetes. Mycological Research 108: 983–1002. http://dx.doi.org/10.1017/S0953756204000851

Li HJ. 2013. Taxonomy and phylogeny of *Trametes* and related genera in China. PhD thesis, Beijing Forestry University. Beijing.

Liu YL, Whelen S, Hall BD. 1999. Phylogenetic relationships among ascomycetes: evidence from an RNA polymerase II subunit. Molecular Biology and Evolution 16: 1799–1808. http://dx.doi.org/ 10.1093/oxfordjournals.molbev.a026092

Matheny PB. 2005. Improving phylogenetic inference of mushrooms with RPB1 and RPB2 nucleotide sequences (*Inocybe*; *Agaricales*). Molecular Phylogenetics and Evolution 35: 1–20. http://dx.doi.org/ 10.1016/j.ympev.2004.11.014

Miettinen O, Rajchenberg M. 2012. *Obba* and *Sebipora*, new polypore genera related to *Cinereomyces* and *Gelatoporia* (*Polyporales*, *Basidiomycota*). Mycological Research 11: 131–147. http://dx.doi.org/ 10.1007/s11557-010-0736-8

Murrill WA. 1905. The *Polyporaceae* of North America 11. A synopsis of the brown pileate species. Bulletin of the Torrey Botanical Club 32: 353–371.

Murrill WA. 1908. *Polyporaceae*. North American Flora 9(2): 73–132.

Niemelä T, Kotiranta H, Penttilä R. 1992. New records of rare and threatened polypores in Finland. Karstenia 32: 81–94.

Núñez M, Ryvarden L. 2001. East Asian polypores 2. *Polyporaceae* s. lato. Synopsis Fungorum 14: 170–522.

Nylander JAA. 2004. MrModeltest 2.2. Program distributed by the author. Evolutionary Biology Centre, Uppsala University.

Petersen JH. 1996. Farvekort. The Danish Mycological Society's colour-chart. Foreningen til Svampekundskabens Fremme, Greve. 6 p.

Posada D, Crandall KA. 1998. MODELTEST: testing the model of DNA substitution. Bioinformatics 14: 817–818.

Posada D, Crandall KA. 2001. Selecting the best-fit model of nucleotide substitution. Systematic Biology 50: 580–601. http://dx.doi.org/ 10.1080/106351501750435121

Ronquist F, Huelsenbeck JP. 2003. MRBAYES 3: Bayesian phylogenetic inference under mixed models. Bioinformatics 19: 1572–1574. http://dx.doi.org/ 10.1093/bioinformatics/btg180

Ryvarden L, Gilbertson RL. 1993. European polypores. Synopsis Fungorum 6: 1–741.

Swofford DL. 2002. PAUP: Phylogenetic analysis using parsimony, version 4.0b10. Sinauer Associates, Inc. Publishers, Sunderland.

Wang B, Dai YC, Cui BK, Du P, Li HJ. 2009. Wood-rotting fungi in eastern China 4. Polypores from Dagang Mountains, Jiangxi Province. Cryptogamie, Mycologie 30: 233–241.

Wang B, Cui BK, Li HJ, Du P, Jia BS. 2011. Wood-rotting fungi in eastern China 5. Polypore diversity in Jiangxi Province. Annales Botanici Fennici 48: 237–246. http://dx.doi.org/10.5735/085.048.0304

White TJ, Bruns TD, Lee S, Taylor J. 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. 315–322, in: MA Innis et al. (eds). PCR protocols, a guide to methods and applications. Academic, San Diego.

ISSN (print) 0093-4666 © 2014. Mycotaxon, Ltd. ISSN (online) 2154-8889

MYCOTAXON

http://dx.doi.org/10.5248/129.21

Volume 129(1), pp. 21–23 *July–September 2014*

A new species of *Uromyces* from Turkey

Zeliha Bahcecioglu

Department of Biology, Faculty of Science and Art, Inonu University, TR 44280, Malatya, Turkey

*Correspondence to: *zeliha.bahcecioglu@inonu.edu.tr*

Abstract — A new species of *Uromyces* on *Myosotis* sp. (*Boraginaceae*) is described from Turkey. As far as is known, no *Uromyces* has previously been described on this host genus. *Uromyces myosotidis* sp. nov. is described and illustrated in this paper.

Key words —Anatolia, new taxon, *Puccineaceae*, *Basidiomycota*

Introduction

Among the approximately 351 species of rust fungi recorded for Turkey, 74 species and one variety of *Uromyces* have been reported (Bahcecioglu & Kabaktepe 2012). Recently, an unknown *Uromyces* specimen was collected in the country on *Myosotis;* it is described here as a new species, *U. myosotidis*.

Materials & methods

The host specimen was collected from Adıyaman Province in 2012, prepared according to established herbarium techniques, and identified from the relevant botanical literature (Davis 1965, 1978; Davis et al. 1988; Grau 1978). Spores were scraped from the dried host specimen and mounted in lactophenol. The preparations were examined with an Olympus CX31 light microscope. Analysis LS Starter software was used to measure at least 30 spores for each spore state. The specimen is preserved in the herbarium of Inonu University (INU), Turkey.

Taxonomy

Uromyces myosotidis Bahç. **sp. nov.** Fig. 1

MycoBank 804880

Differs from *Uromyces tairae* by its smaller teliospores and urediniospores.

Type: Turkey, Adıyaman Province, 10–12 km between Adıyaman and Celikhan, 1500–1600 m, on *Myosotis* sp. (*Boraginaceae*), 6 June 2012, Zeliha Bahcecioglu 3888 (**Holotype**, INU).

Etymology: from *Myosotis,* the host genus.

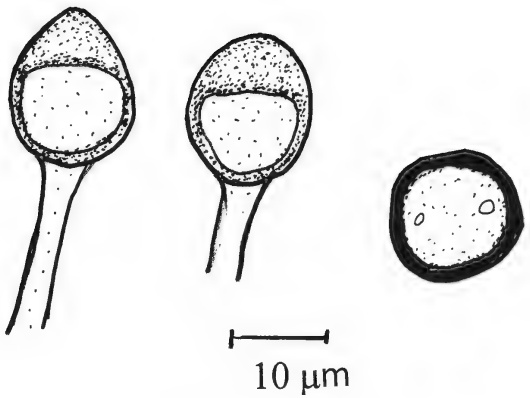


Fig. 1. *Uromyces myosotidis* (holotype). Teliospores and urediniospore.

Pycnia and aecia not seen. Uredinia and telia often mixed together, hypophyllous, covered by epidermis, small, scattered, brown. Urediniospores 14–16 × 13–16 µm globoid, subgloboid, walls 1.5–2 µm thick, brown, smooth or finely sparsely echinulate, with 2–3 equatorial pores. Teliospores 14–22 × 12–18 µm, globoid, subgloboid; walls ≤5–8 µm apiculus, dark brown, smooth. Pedicels ≤56 µm long, broken, hyaline.

Discussion

As far as is known, no *Uromyces* has previously been described on *Myosotis*. *Uromyces myosotidis* differs from *Uromyces* spp. determined on other genera of *Boraginaceae* in teliospore and urediniospore form and size. *Uromyces tairae* Hirats. f. on *Messerschmidia* (= *Tournefortia*) has larger teliospores (25–38 × 20–28 µm) and urediniospores (22–30 × 17–25 µm; Hiratsuka 1940). *Uromyces permeritus* Cummins and *U. dolichosporus* Dietel & Holw. on *Tournefortia* have ellipsoid, long fusiform, or long clavate teliospores (Holway 1897; Cummins 1940). *Uromyces heliotropii* Sred. on *Heliotropium* has larger teliospores (21–25 × 18–22 µm) with an apical pore (Ul'yanishchev et al. 1985; Kuprevich & Ul'yanishchev 1975).

Acknowledgments

This work was supported by the BAP of Inonu University, project numbers 2010/104. Thus many thank BAP of Inonu University, also thanks Prof. Dr. Bayram Yıldız Balıkesir University, Balıkesir (Turkey), for help with identification of host and Dr. Mehmet Candan for presubmission expert reviews and Dr. Brian Spooner (Kew garden) for helpful suggestions.

Literature cited

Bahcecioglu Z, Kabaktepe S. 2012. Checklist of rust fungi in Turkey. Mycotaxon 119: 494. http://www.mycotaxon.com//resources/checklists/Bahcecioglu-v119-checklist.pdf

Cummins GB. 1940. *Uredinales* of New Guinea. Mycologia 32: 32–375. http://dx.doi.org/10.2307/3754317

Davis PH. 1965. Flora of Turkey and the East Aegean Islands. Vol. 1. Edinburgh. University Press.

Davis PH. 1978. Flora of Turkey and the East Aegean Islands. Vol. 6. Edinburgh. University Press.

Davis PH, Mill RR, Tan K. 1988. Flora of Turkey and the East Aegean Island. Vol. 10 (Suppl.). Edinburgh: University Press.

Grau J. 1978. *Myosotis* L. 264-280, in: PH Davis (ed.). Flora of Turkey and the East Aegean Islands. Vol. 6. Edinburgh: University Press.

Hiratsuka N. 1940. *Uredinales* collected in Korea. Bot. Mag. Tokyo 54: 427–432.

Holway EWD. 1897. Mexican Fungi. Bot. Gaz. 24: 23–38. http://dx.doi.org/10.1086/327549

Kuprevich VF, Ul'yanishchev VI. 1975. The key for determination of rust fungi of the USSR. Minsk. [in Russian]

Ul'yanishchev VI, Babayan DN, Melia MS. 1985. Key to the rust fungi of Transcaucasia. Baku. [in Russian]

ISSN (print) 0093-4666 © 2014. Mycotaxon, Ltd. ISSN (online) 2154-8889

MYCOTAXON

http://dx.doi.org/10.5248/129.25

Volume 129(1), pp.25–31 *July–September 2014*

Cladosporium species from hypersaline environments as endophytes in leaves of *Cocos nucifera* and *Vitis labrusca*

Rafael José Vilela de Oliveira*[1], Thaís Emanuelle Feijó de Lima, Gladstone Alves da Silva, & Maria Auxiliadora de Queiroz Cavalcanti*[2]

Departamento de Micologia, Universidade Federal de Pernambuco, Rua Nelson Chaves, s/n, Cidade Universitária, Recife, 50670-901, Brazil

* Correspondence to: [1] *rafaelvilela87@gmail.com*, [2] *xiliamac@gmail.com*

Abstract — *Cladosporium dominicanum* and *C. halotolerans* were isolated in Pernambuco, Brazil, from healthy leaves of *Cocos nucifera* and *Vitis labrusca,* respectively. This is the first report of these species as endophytes and the first record of *C. dominicanum* for Brazil.

Key words —ITS, phylogenetic analysis, tropical plants

Introduction

Cladosporium was originally described by Link in 1816, and *Cladosporium herbarum* was selected from amongst Link's original species by Clements & Shear (1931) as the generic type (see also Bensch et al. 2010). With over 772 taxa, *Cladosporium* is one of the largest hyphomycete genera (Dugan et al. 2004). Bensch et al. (2012), who analyzed the genus phylogenetically, created an identification key and described 169 species. *Cladosporium* species are commonly found as plant and human pathogens and are also decomposers of food, paints, textiles, and organic matter (Ellis, 1971, 1976, Kwon et al. 2001; Liu et al. 2001). They occur as endophytes in plants of tropical regions (Costa et al. 2012) and are the most frequent endophytic fungi in some plants (Bezerra et al. 2012).

Cladosporium halotolerans is saprobic on various substrates and has been isolated in hypersaline water from subtropical regions, indoor environments, arctic ice, human and animal lesions, plants, rock, window frames, and mycorrhizal roots (Bensch et al. 2012). *Cladosporium dominicanum* was first isolated from hypersaline water and was also found as a saprobe on the surface of fruits (Bensch et al. 2012).

Here we report the first records of *C. dominicanum* as an endophyte in leaves of *Cocos nucifera* (and its first occurrence from Brazil) and of *C. halotolerans* as an endophyte in the leaves of *Vitis labrusca*.

Materials & methods

During February 2010 (dry season), healthy mature leaves of *Vitis labrusca* were collected from forest areas of the municipalities of São Vicente Férrer, Pernambuco, and in May 2012 (dry season), healthy mature leaves of *Cocos nucifera* were collected from forest areas of the municipalities of Goiana, Pernambuco.

Sterilization, isolation, and identification

In the laboratory, each leaf was washed gently in running water and soap. Leaf discs were cut with a sterile metallic cork punch (6 mm diam.), decontaminated with 70% alcohol for 30 sec and sodium hypochlorite solution (NaOCl) at 2% for 2.5 min, and twice washed with sterilized distilled water in order to remove the hypochlorite excess (Petrini 1996; modified technique). Six surface sterilized discs were transferred in triplicate to each Petri dish containing malt extract agar (MEA) + chloramphenicol (50 $mg.L^{-1}$) to prevent bacterial growth. The plates were incubated at room temperature (28 ± 2°C) and observed daily during 15 days for colony development. For asepsis control, 50 µL of water, used to remove hypochlorite, was plated in MEA to confirm surface disinfection (Pereira et al. 1993). Species identification was based on macro- and microstructural characteristics of the colony, according to Bensch et al. (2012). For each species (*Cladosporium halotolerans* and *C. dominicanum*), one isolate was deposited in the URM Culture Collection of the Universidade Federal de Pernambuco.

Molecular analyses

The fungi biomass was obtained from cultures grown on malt agar contained in test tubes and kept at 28°C for up to six days. All mycelium was removed from the test tube with the aid of a platinum loop; the material was transferred to 2 ml micro-tubes with screw caps to which were added 0.5 g of glass beads with two different diameters in the 1:1 ratio (acid-washed, 150–212 µm and 425–600 µm; Sigma, U.S. sieve). The material was crushed by stirring at high speed in a FastPrep.

Genomic DNA was extracted according to Góes-Neto et al. (2005). The material was washed with chloroform : isoamyl alcohol (24:1) and following homogenization of the material in CTAB buffer at 2%, besides isopropanol precipitation, washing in 70% ethanol, and re-suspended in 50 µL of ultrapure water.

Primers ITS1 and ITS4 (White et al. 1990) were used to amplify the ITS region. PCR reactions were carried out in 50 µL volumes containing 75 mM Tris-HCl pH 8.8, 200 mM $(NH_4)_2SO_4$, 0.01% Tween 20, 2 mM $MgCl_2$, 200 µM each dNTPs, 1 µM of each primer, and 2 units of Taq DNA polymerase (Fermentas, Maryland, USA); cycling parameters were 5 min at 95°C (1 cycle), 45s at 94°C, 1 min at 60°C, 1 min at 72°C (39 cycles), and a final elongation of 7 min at 72°C.

The final amplicons were purified with the PureLink PCR Purification Kit (Invitrogen). Sequencing was provided by the Human Genome Research Center (São Paulo, Brazil). Sequence data were compared to gene libraries (EMBL and GenBank) using BLASTn. The new sequences deriving from the species were deposited in the NCBI database under the accession numbers KJ000286 and KJ000287.

Phylogenetic analyses

The phylogeny was reconstructed by sequences of the ITS1+5.8s+ITS2 rDNA gene. The fungal sequences were aligned in ClustalX (Larkin et al. 2007) and edited with the BioEdit program (Hall 1999). Prior to phylogenetic analysis, the model of nucleotide substitution was estimated using Topali 2.5 (Milne et al. 2004). Bayesian analysis (two runs over 1×10^6 generations with a burnin value of 2500) were performed in MrBayes 3.1.2 (Ronquist & Huelsenbeck 2003), and maximum likelihood analysis (1000 bootstrap) was performed in PhyML (Guindon & Gascuel 2003), launched from Topali 2.5, using the GTR + I + G model. Neighbor-joining (established with the models cited above) and maximum parsimony analyses were performed using PAUP*4b10 (Swofford 2003) with 1000 bootstrap replications.

The phylogenetic tree described in Zalar et al. (2007) was used as reference.

Results

Taxonomy

Cladosporium halotolerans Zalar, de Hoog & Gunde-Cim., Stud. Mycol. 58: 172. 2007.

Potato Dextrose Agar: Colonies after 14 days of culture at 25°C, presenting a growth of 53 mm, dark grayish green, reverse dark green. Conidiophores erect, lateral or terminal, olive to brown, septate, smooth, branched, 10–77.5 × 2.5–5 µm. Conidia in tandem, smooth, olive to brown, non-septate, globose, subglobose, or shortly limoniform (majority), slightly rough, 1.25–3.75 × 2.5–3.75 µm. Conidiogenous cells undifferentiated. Branch-primary conidia rare, with ≤2 distal scars; branch-secondary conidia, 0–1 septum (no majority), with ≤3 distal scars, 7.5–27.5 × 2.5–3.75 µm.

Malt Agar: Colonies after 14 days of culture at 25°C, presenting a growth of 59 mm, dark green grayish yellowish to dark green, reverse dark green. Conidiophores erect, lateral or terminal, olive to brown, septate, smooth, branched, 13–190 × 2.5–6.25 µm. Conidia in tandem, smooth, olive to brown, non-septate, globose to subglobose, 2.5–5 × 1.25–2.5 µm. Conidiogenous cells undifferentiated. Branch-primary conidia (2.5 × 2.5–3 µm) with up to 2 distal scars; branch-secondary conidia, 0–1 septum (no majority), with ≤2–3 distal scars, 3–22.5 × 2.5–3.75 µm.

Malt + 5% NaCl: Colonies after 14 days of culture at 25°C, presenting a growth of 50 mm, dark grayish green, reverse dark green, and with little sporulation. Conidiophores erect, lateral or terminal, olive to brown, septate, smooth, branched, 15–212.5 × 2.5 µm. Conidia in tandem, smooth, olive to brown, non-septate, globose (majority) to limoniform, rough 2.5–5 × 1.25–3.75 µm. Conidiogenous cells undifferentiated. Branch-primary conidia present; branch-secondary conidia, 0–1 septum, with up to 3 (mostly 2) distal scars, 10–25 × 3.75 µm.

Specimen examined: **BRAZIL. Pernambuco:** São Vicente Férrer, in healthy mature leaves of *Vitis labrusca* L. cv. Isabel, Feb 2010, T.E.F. Lima (URM6963; GenBank KJ000286).

Notes: *Cladosporium halotolerans* is known from Africa, Arctic, Asia, Australasia, Europe, North America, and Central and South America. This is the first recorded occurrence of the species as an endophyte.

Cladosporium dominicanum Zalar, de Hoog & Gunde-Cim., Stud. Mycol. 58: 169. 2007.

Potato Dextrose Agar: Colonies after 14 days of culture at 25°C, presenting a growth of 31 mm, green olive, velvety, reverse dark gray. Conidiophores erect, lateral or terminal, olive to brown, slightly verrucose, septate, branched or unbranched, 36–245 × 2–2.5 µm. Conidiogenous cells undifferentiated. Conidia in tandem, slightly verrucose, light brown, not septate, ovoid, 3.7–5.58 × 2.7–3.2 µm. Ramoconidia rarely formed; secondary cylindrical ramoconidia, 0–1 septate 14–28 × 2.5–3 µm.

Malt agar: Colonies after 14 days of culture at 25°C, presenting a growth of 34 mm, dark green, velvety, grooved, reverse dark gray. Conidiophores erect, lateral or terminal, olive to brown, slightly verrucose, septate, branched or unbranched, 50–160 × 2–2.5 µm. Conidiogenous cells undifferentiated. Conidia in tandem, slightly verrucose, light brown, non-septate, ovoid, 3.7–5.58 × 2.7–3.2 µm. Ramoconidia rarely formed; secondary cylindrical ramoconidia, 0–1 septate, 12–19 × 2.5–3 µm.

Malt + 5% NaCl: Colonies after 14 days of culture at 25°C, presenting a growth of 40 mm, light green, grooved, reverse dark gray. Conidiophores erect, lateral or terminal, olive to brown, slightly verrucose, septate, branched or unbranched, 45–107.5 × 2–2.5 µm. Conidiogenous cells undifferentiated. Conidia in tandem, slightly verrucose, light brown, non-septate, ovoid, 3.7–4.8 × 2.7–3.2 µm. Ramoconidia rarely formed, secondary cylindrical ramoconidia, 0–1 septate, 10–22.5 × 2.5–3 µm.

Specimen examined: **BRAZIL. Pernambuco:** Goiana, in healthy mature leaves of *Cocos nucifera* L., May 2012, R.J.V. Oliveira (URM6962; GenBank KJ000287).

Notes: *Cladosporium dominicanum* was previously known from Asia (Iran) and Central America (Dominican Republic). This is the first recorded occurrence of the species for South America (Brazil) and as an endophyte.

Molecular & phylogenetic analysis

The sequences generated from our fungal isolates grouped firmly in *Cladosporium*. Our isolate URM6962 (from leaves of *Cocos nucifera*) formed a clade with sequences from *C. dominicanum*, and isolate URM6963 (from leaves of *Vitis labrusca*) formed a clade with sequences from *C. halotolerans* (Fig. 1).

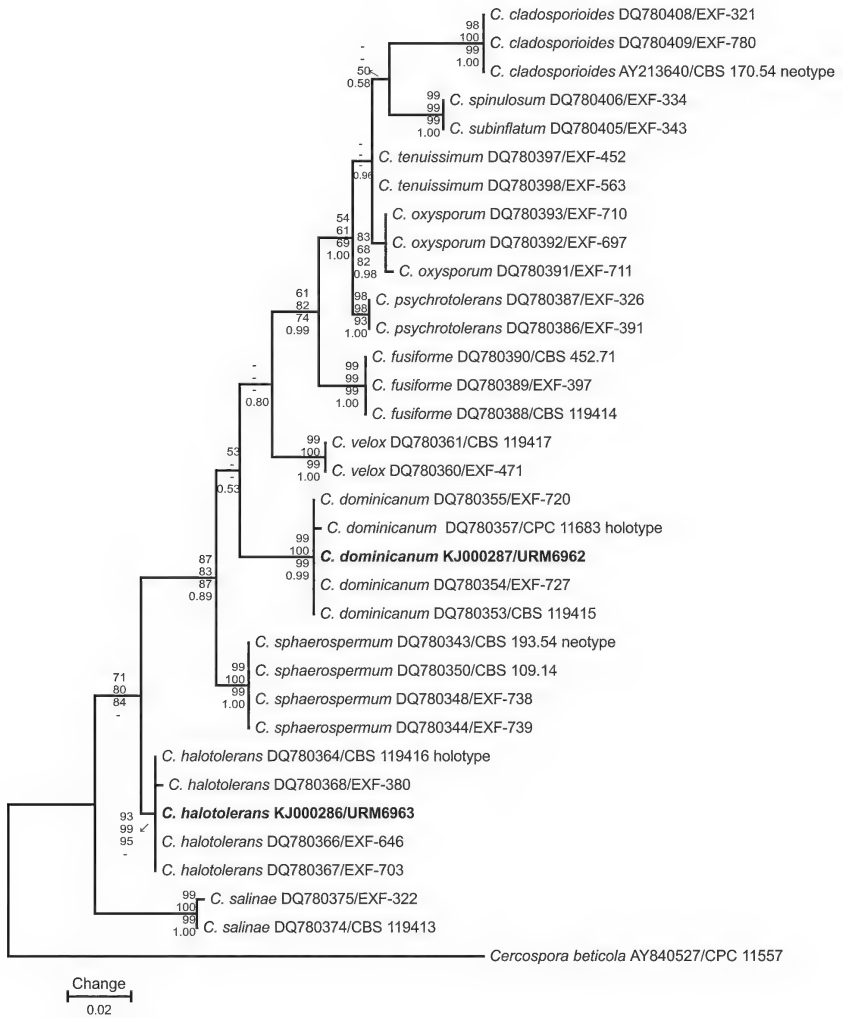


FIG. 1. Phylogenetic reconstruction of the sphaerospermum complex in *Cladosporium* obtained from sequences of the ITS region. Sequences are labeled with their GenBank accession numbers. Support values (from top) are from neighbor-joining (NJ), maximum parsimony (MP), maximum likelihood (ML) and Bayesian analyses. Only bootstrap values of at least 50% are shown.

In their review of *Cladosporium,* Bensch et al. (2012) showed that *C. halotolerans* and *C. dominicanum* belong to the *C. sphaerospermum* complex and are grouped close together.

In the Blastn analysis, our *C. dominicanum* sequence (KJ000287) showed 100% identity with sequence DQ780353 of the *C. dominicanum* ex-holotype culture (EXF752 = CBS 119415) and with two sequences (KC763352, KC845931) from Chinese isolates labelled "*C. sphaerospermum*". However, as sequence DQ780343 of the *C. sphaerospermum* neotype (CBS 193.54) showed only 97% identity with the sequence of our isolate URM6962, we conclude that sequences KC763352 and KC845931 in fact represent *C. dominicanum*.

In the Blastn analysis, our *C. halotolerans* sequence (KJ000286) showed 100% identity with sequence DQ780364 of the *C. halotolerans* ex-holotype culture (EXF572 = CBS 119416) and with 24 databank sequences labelled either "*C. cladosporioides*" (EF577236, AY361968, AB456576) or "*C. sphaerospermum*" (AB572909, AB572908, AB572903, AB572897, JN084018, AY625063, AM182174, AM182171, AM182168, AM176749, AM176685, EU759978, EU823317, JX156365, KC009836, JX839460, HQ263345, HQ248189, GU017501, JN253512, JN253512). However as sequence HM148003 of the *C. cladosporioides* neotype (CBS 17054) showed only 98% identity and the *C. sphaerospermum* neotype sequence showed only 96% identity with the sequence of our isolate URM6963, we conclude that the 24 database sequences probably represent *C. halotolerans* and not *C. cladosporioides* or *C. sphaerospermum*.

Acknowledgments

The authors thank Dr. José Luiz Bezerra and Dr. João Lúcio Azevedo for presubmission critical review. Special acknowledgements to Dr. Shaun Pennycook and Dr. Lorelei Norvell for their additional important corrections and suggestions to the manuscript. The authors also acknowledge the financial support provided by Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq).

Literature cited

Bensch K, Groenewald JZ, Dijksterhuis J, Starink-Willemse M, Andersen B, Shin H-D, Dugan FM, Schroers H-J, Braun U, Crous PW. 2010. Species and ecological diversity within the *Cladosporium cladosporioides* complex (*Davidiellaceae, Capnodiales*). Studies in Mycology 67. 96 p. http://dx.doi.org/10.3114/sim.2010.67.01

Bensch K, Braun U, Groenewald JZ, Crous P.W. 2012. The genus *Cladosporium*. Studies in Mycology 72. 401 p. http://dx.doi.org/10.3114/sim0003

Bezerra JDP, Santos MGS, Svedese VM, Lima DMM, Fernandes MJS, Paiva LM, Souza-Motta CM. 2012. Richness of endophytic fungi isolated from *Opuntia ficus-indica* Mill. (*Cactaceae*) and preliminary screening for enzyme production. World Journal of Microbiology and Biotechnology 28: 1989–1995. http://dx.doi.org/10.1007/s11274-011-1001-2

Clements FE, Shear CL. 1931. The genera of fungi. New York: H.W. Wilson Co. 496 p.

Costa IPMW, Assunção MMC, Lima TEF, Oliveira RJV, Cavalcanti MAQ. 2012. Checklist of endophytic fungi from tropical regions. Mycotaxon 119: 493. http://www.mycotaxon.com/resources/checklists/costa_v119_checklist.pdf

Dugan FM, Schubert K, Braun U. 2004. Check-list of *Cladosporium* names. Schlechtendalia 11. 103 p.

Ellis MB. 1971. Dematiaceous hyphomycetes. Commonwealth Mycological Institute, Kew. England. 608 p.

Ellis MB. 1976. More dematiaceous hyphomycetes. Commonwealth Mycological Institute, Kew. England. 507 p.

Góes-Neto A, Loguercio-Leite C, Guerrero RT. 2005. DNA extraction from frozen field-collected and dehydrated herbarium fungal basidiomata: performance of SDS and CTAB-based methods. Biotemas 18: 19–32.

Guindon S, Gascuel O. 2003. A simple, fast, and accurate algorithm to estimate large phylogenies by maximum likelihood. Systematic Biology 52(5): 696–704. http://dx.doi.org/10.1080/10635150390235520

Hall TA. 1999. BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. Nucleic Acids Symposium Series 41: 95–98.

Kwon JH, Kang SW, Kim JS, Park CS. 2001. Scab of tea (*Thea sinensis*) caused by *Cladosporium herbarum* in Korea. Plant Pathology Journal 17: 350–353.

Larkin MA, Blackshields G, Brown NP, Chenna R, McGettigan PA, McWilliam H, Valentin F, Wallace IM, Wilm A, Lopez R, Thompson JD, Gibson TJ, Higgins DG. 2007. Clustal W and Clustal X version 2.0. Bioinformatics 23: 2947–2948. http://dx.doi.org/10.1093/bioinformatics/btm404

Link HF. 1816. Observationes in ordines plantarum naturales III. Magazin der Gesellschaft Naturforschender Freunde Berlin 7: 37–38.

Liu CH, Zou WX, Lu H, Tan RX. 2001. Antifungal activity of *Artemisia annua* endophyte against phytopathogenic fungi. Journal of Biotechnology 88(3): 277–282. http://dx.doi.org/10.1016/S0168-1656(01)00285-1

Milne I, Wright F, Rowe G, Marshall DF, Husmeier D, McGuire G. 2004. TOPALi: Software for automatic identification of recombinant sequences within DNA multiple alignments. Bioinformatics 20: 1806–1807. http://dx.doi.org/10.1093/bioinformatics/bth155

Pereira JO, Azevedo JL, Petrini O. 1993. Endophytic fungi of *Stylosanthes*. Mycologia 85: 362–364. http://dx.doi.org/10.2307/3760696

Petrini O. 1996. Ecological and physiological aspects of host specific and endophytic fungi. 87–100, in: SC Redlin, LM Carris (Eds). Endophytic fungi in grasses and woody plants. St Paul: American Phytopathological Society Press.

Ronquist F, Huelsenbeck JP. 2003. MrBayes 3: Bayesian phylogenetic inference under mixed models. Bioinformatics 19(12): 1572–1574. http://dx.doi.org/10.1093/bioinformatics/btg180

Sutton BC.1991. Notes on deuteromycetes III. Sydowia 43: 264–280.

Swofford DL. 2003. PAUP*. Phylogenetic Analysis Using Parsimony (* and other methods), Version 4. Sinauer Associates, Sunderland, Massachusetts.

White TJ, Bruns T, Lee S, Taylor L. 1990. Amplification and direct sequencing of fungal RNA genes for phylogenetics. 315–322, in MA Innis et al. (eds). PCR protocols. a guide to methods and applications. Academic Press, San Diego.

Zalar P, Hoog GS de, Schroers H-J, Crous PW, Groenewald JZ, Gunde-Cimerman N. 2007. Phylogeny and ecology of the ubiquitous saprobe *Cladosporium sphaerospermum*, with descriptions of seven new species from hypersaline environments. Studies in Mycology 58: 157–183. http://dx.doi.org/10.3114/sim.2007.58.06

ISSN (print) 0093-4666 © 2014. Mycotaxon, Ltd. ISSN (online) 2154-8889

MYCOTAXON

http://dx.doi.org/10.5248/129.33

Volume 129(1), pp. 33–40 *July–September 2014*

Aschersonia narathiwatensis sp. nov. from southern Thailand

Suchada Mongkolsamrit[1*], Artit Khonsanit[1], Wasana Noisripoom[1], Pagmadulam Baldorj[2], & J. Jennifer Luangsa-ard[1]

[1]*BIOTEC, NSTDA Science Park,*
113 Paholyothin Road, Klong 1, Klong Luang, Pathum Thani, Thailand
[2]*Institute of Biology, Mongolian Academy of Sciences, Ulaanbaatar-51, Mongolia*
* Correspondence to: *suchada@biotec.or.th*

Abstract — *Aschersonia narathiwatensis* was isolated from whitefly nymphs (*Hemiptera*) collected at Hala-Bala Wildlife Sanctuary in Narathiwat Province, Thailand. This species has circular flattened-discoid brown to dark brown stroma and non-fragmenting ascospores. A comparison of macro-and microscopic characters among similar species is given. Phylogenetic analyses of nuclear ribosomal large subunit (LSU) and translation elongation factor 1-α (tef-1α) support recognizing this fungus as a new species.

Key words — morphology, phylogenetics, taxonomy

Introduction

Aschersonia Mont. is a genus of entomopathogenic fungi that attack scale insects (*Coccidae, Hemiptera*) or white flies (*Aleyrodidae, Hemiptera*) of which several species are reported from tropical and subtropical regions (Montagne 1848; Petch 1921; Mains 1959a,b; Chaverri et al. 2008; Mongkolsamrit et al. 2009, 2011; Qiu et al. 2009, 2010; Qiu & Guan 2010). Research on entomopathogenic fungi has been conducted under the biodiversity studies program to further our knowledge of these fungi in Thailand. Here we describe a new *Aschersonia* species from a tropical rainforest in southern Thailand.

Materials & methods

Surveys and collections were made during the rainy season in Hala-Bala Wildlife Sanctuary, which is located at the southernmost tip of peninsular Thailand on the international border with Malaysia. Pure cultures were made from isolations from the teleomorphic state following Mongkolsamrit et al. (2009). Sections of the stroma prepared by using a freezing microtome were mounted in distilled water and in cotton blue in lactophenol (Heritage et al. 1996). Observation of the microscopic characters

(e.g., perithecia, ascospores and asci) were made using a light microscope. The color of fresh specimens and cultures were compared with the colors from standard code of Kornerup & Wanscher (1962). A voucher specimen and culture were deposited in BIOTEC Bangkok Herbarium (BBH) and BIOTEC Culture Collection, Thailand.

Genomic DNA was extracted from each sample with cetyltrimethyl-ammonium bromide (CTAB) according to Mongkolsamrit et al. (2009). PCR amplification was done

Table 1. List of fungi used in molecular analyses.
(Newly generated sequences set in bold.)

Species	Voucher/Isolate	GenBank	
		LSU	EF1-α
Aschersonia badia	BBH 14255 = BCC 8105	DQ518752	**KC713630**
	BBH 32386 = BCC 55524	—	**KF016991**
A. calendulina	*BBH 17344 = BCC 20309	GU552154	**KF016993**
	BBH 17325 = BCC 20306	GU552148	**KF016994**
	*BCC 9483	DQ384938	DQ384962
A. luteola	*BBH 13011 = BCC 19360	GU552155	GU552147
	BBH 14277 = BCC 9481	DQ384945	DQ384974
	BBH 14078 = BCC 7865	DQ384946	DQ384975
A. minutispora	BBH 17373 = BCC 20635	GU552149	DQ552143
	BBH 14143 = BCC 7959	GU552150	—
	BBH 12951 = BCC 17487	GU552151	GU552144
A. narathiwatensis	*BBH 31120 = BCC 49495	**KC713633**	**KC713628**
	*BBH 31120 = BCC 49498	**KF016998**	—
	*BBH 31120 = BCC 49499	**KF016999**	**KF016992**
A. samoensis	BBH 14540 = BCC 8775	DQ384944	DQ384972
	*BCC 8237	DQ384937	DQ384977
	*NHJ 4093	AF327381	—
Balansia henningsiana	GAM 16112	AY489715	AY489610
Epichloe elymi	C. Schardl 760	AY986924	AY986951
Moelleriella phyllogena	P.C. 554 = CUP 067784	EU392609	EU392673
	P.C. 555 = CUP 067785	EU392610	EU392674
M. raciborskii	I93-901b = ARSEF 7661	EU392611	EU392676
M. umbospora	P.C. 457 = CUP 067816	AY986904	AY986929
Samuelsia geonomis	P.C. 614 = CUP 067857	EU392638	EU392692
S. mundiveteris	BBH 24706 = BCC 31750	GU552157	GU552142
	BBH 26961 = BCC 40021	GU552152	GU552145
	BBH 26961 = BCC 40022	GU552153	GU552146
S. rufobrunnea	P.C. 613 = CUP 067858	AY986918	AY986944

* Living cultures isolated from ascospores.

in 50 µl volumes consisting of 1× PCR buffer, 200 µM of each of the four dNTPs, 2.5 mM MgCl2, 1 U Taq DNA polymerase (Promega, Madison, Wisconsin), and 0.5 µM of each primer. The amplification of the partial gene region of the large subunit nuclear ribosomal DNA (LSU) used primers LRORf and LR5r (Vilgalys & Sun 1994) and the translation elongation factor 1-α (TEF-1α) was amplified with primers EF-1a: 983f (Carbone & Kohn 1999) and 2218r (Rehner 2001). After amplification in a MJ Research

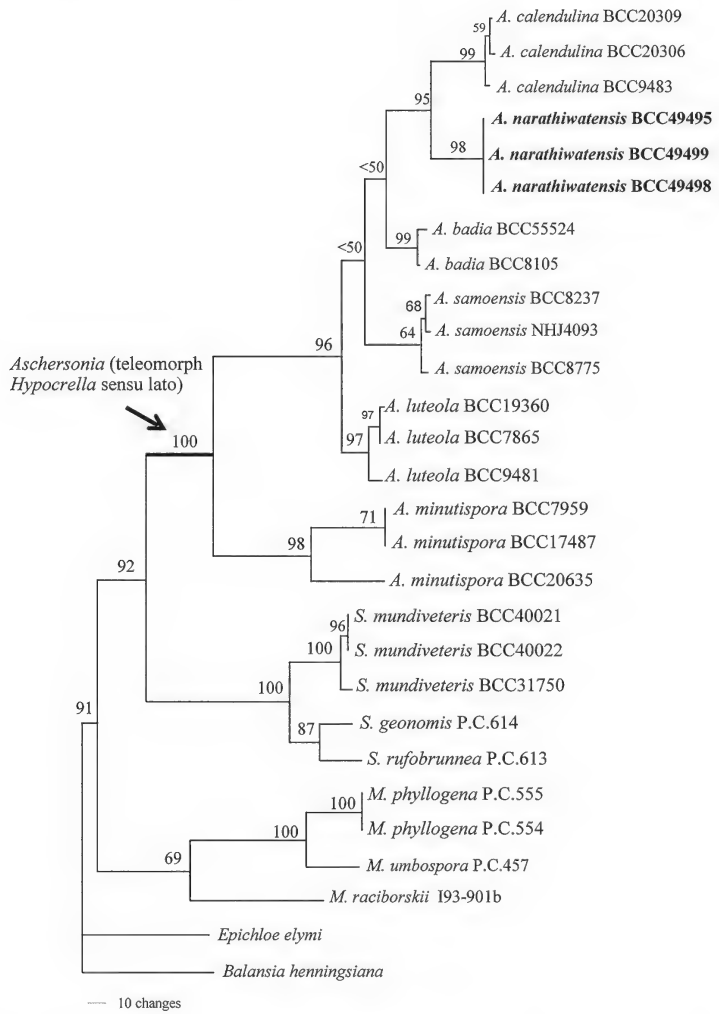


PLATE 1. Phylogenetic relationship of *Aschersonia narathiwatensis* and related species based on maximum parsimony analysis of the LSU and EF-1 α. Numbers above each branch represent bootstrap support from 1000 replicates. [*S.* = *Samuelsia; M.* = *Moelleriella*]

DNA Engine ALD1244 thermal cycler following the procedure described in Sung et al. (2001), the PCR products were purified with a QIAquick PCR Purification Kit (Qiagen GmbH, Hilden, Germany), following the manufacturer's instructions. Purified PCR products were sent to Macrogen Inc. Korea for sequencing. Sequences were proofread manually and assembled using BioEdit v. 7.0.4 (Hall 1999), and deposited in GenBank (Table 1). Sequences were aligned with Clustal W incorporated in BioEdit, and alignments were refined manually by direct examination. Maximum parsimony analysis was performed on the combined dataset of LSU and *tef*-1α sequences in PAUP 4.0b10 using random addition sequence (10 replications) where gaps were treated as missing data. Bootstrap analysis was performed using maximum parsimony criterion in 1000 replication samples. *Balansia henningsiana* GAM 16112 and *Epichloe elymi* C. Schardl 760 were used as outgroup taxa.

Results

Molecular analysis

LSU sequences were obtained from three isolates, and *tef*-1α sequences from six isolates. Twenty-four LSU-related sequences and 19 TEF-1α-related sequences from GenBank were used to construct a phylogenetic tree. Of the 1652 characters in the combined alignment, 268 characters were parsimony informative. Maximum parsimony analyses of this data set yielded one parsimonious tree (tree length 698; CI = 0.610, RI = 0.797, RC = 0.486, HI = 0.390) as shown in Plate 1. The phylogenetic tree shows that three teleomorphic isolates assigned to our new species (BCC 49495, BCC 49498 and BCC 49499) belong to the genus *Aschersonia* with a strong bootstrap support of 100%.

Taxonomy

Aschersonia narathiwatensis Mongkols., Khonsanit & Luangsa-ard, **sp. nov.**

MycoBank MB 803933 Plate 2

Differs from *A. calendulina* by its brown to dark brown stromata and shorter ascospores.

Type — Thailand: Narathiwat Province, Headquarter Trail, Hala-Bala Wildlife Sanctuary, on whitefly nymphs (*Hemiptera*) on underside of dicotyledonous leaf, 24 Aug. 2011, A. Khonsanit (**Holotype**, BBH 31120; ex-holotype cultures BCC 49495 [GenBank, KC713633, KC713628], BCC 49498 [GenBank, KF016998], BCC 49499 [GenBank, KF016999, KF016992].

Etymology — referring to Narathiwat Province, the collection location.

Teleomorph: *Hypocrella*

Stromata circular, flattened-discoid, brown to dark brown, 5E7, ≤1–3 mm diam and 0.5–1 mm high, margin undulate, base slightly constricted; internally hyaline to whitish-coloured. Hypothallus membranous, slight or thickened, 0.5–1 mm thick, pale yellow. Perithecia crowded, immersed, 200–430 × 100–200 µm, elongate flask-shaped, ostioles slightly projecting, translucent

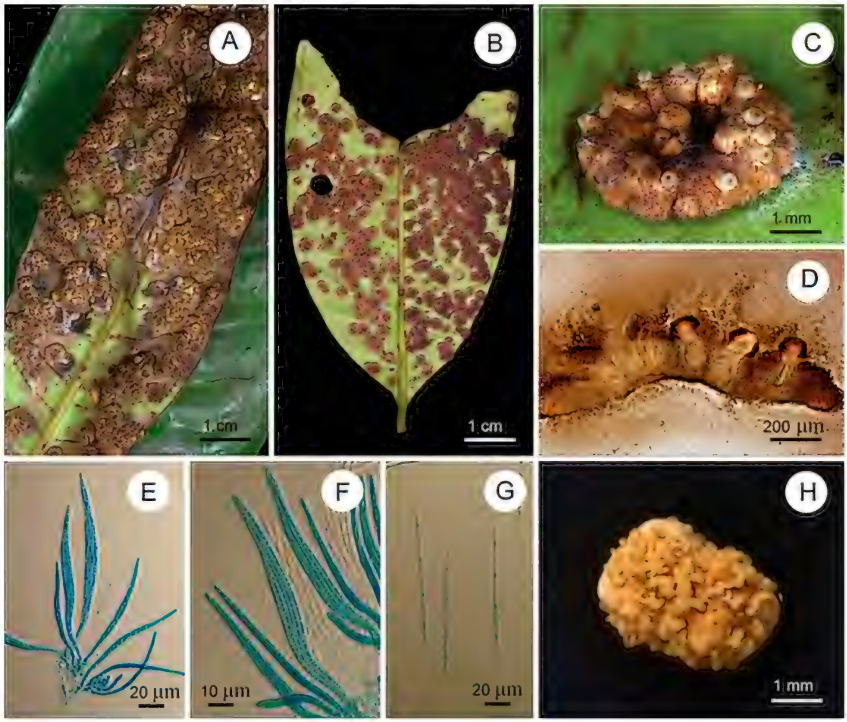


Plate 2. *Aschersonia narathiwatensis*: A–C. Stromata on infected whitefly nymph hosts; D. Cross section of stroma showing perithecia; E, F. Mature ascus with developing asci; G. Whole ascospores; H. Sporulating colony on PDA at 20°C after 4 wk.

dark-brown. Asci 8-spored, ≤200 × 10–12 µm. Ascospores whole, non-fragmenting, 80–100 × 1.5–2 µm, blunt at upper end tapering to base.

Cultural characteristics Ascospores germinating within 24 h on PDA. Colonies on potato dextrose agar (PDA) slow-growing, attaining a diam of 5 mm in 4 wk. Optimal temperature 20–25°C, with no growth at <5°C and >35°C. Stromatic colonies pale brown, forming moderately compact stromata. Conidial masses pale brown, 4C6, appearing as abundant slimy masses from immersed pycnidia scattered over surface.

Comments: In this study, the anamorphic generic name *Aschersonia* is used based on the recent major changes in fungal nomenclature requiring only one scientific name per fungal species (Hawksworth 2011; Taylor 2011; Gams et al. 2012; McNeill et al. 2012). Described earlier in 1848, *Aschersonia* has priority over the teleomorphic generic name, *Hypocrella* Sacc. (1878). In this study, *Hypocrella* is treated as the teleomorph of *Aschersonia*.

Aschersonia narathiwatensis was been found only at Hala-Bala Wildlife Sanctuary in southern Thailand. Despite several attempts to find it in different seasons of the year, the anamorphic state of this species was not found in the field. The teleomorphic characters are presented and compared with other Thai species representing *Hypocrella* sensu lato (Table 2). The teleomorphic state of *A. narathiwatensis* is similar to that of *A. calendulina* reported from Thailand by Mongkolsamrit et al. (2009) in having flattened discoid stromata but differs in the color and size of the stromata. *Aschersonia narathiwatensis* has brown to dark brown stromata while those of *A. calendulina* are bright orange. The ascospores of *A. narathiwatensis* are somewhat shorter than those of *A. calendulina* (Table 2). In the phylogenetic analysis, *A. narathiwatensis* and *A. calendulina* form a clade with 95% support. Both morphological characters and phylogenetic analysis of the partial gene regions of LSU and *tef*-1α support *A. narathiwatensis* as a new species.

Table 2. Morphological comparison of *Aschersonia* teleomorphs in Thailand.

Species	Stromatal shape	Stromatal colour	Asci (µm)	Ascospores (µm)	Reference
A. calendulina	Flattened discoid	Bright orange	<180 × 10–12	130–150 × 1.5–2	Mongkolsamrit et al. (2009)
A. luteola	Discoid to stud-shaped	Yellow	<170 × 7.5–8	75–120 × 2	Mongkolsamrit et al. (2009)
A. narathiwatensis	Flattened discoid	Brown	<200 × 10–12	80–100 × 1.5–2	This study
A. samoensis	Discoid to stud-shaped	Rust orange	140–180 × 8	60–90 × 4–5	Hywel-Jones & Evans (1993)

Acknowledgments

The authors would like to thank Dr. Amy Y. Rossman (Systematic Mycology & Microbiology Laboratory, Beltsville, MD, USA) and Dr. Richard A. Humber (USDA-ARS Biological Integrated Pest Management Research Unit, Ithaca, NY, USA) for their comments and suggestions to improve this manuscript. We also would like to thank Prof. Morakot Tanticharoen, Dr. Kanyawim Kirtikara, and Dr. Lily Eurwilaichitr for their support of the program, 'Biodiversity studies of entomopathogenic fungi in Thailand." We are grateful to Mr. Soonthorn Todam and the staff at Hala-Bala Wildlife Sanctuary for their kind cooperation.

Literature cited

Carbone I, Kohn LM. 1999. Method for designing primer sets for speciation studies in filamentous ascomycetes. Mycologia 91: 553–556. http://dx.doi.org/10.2307/3761358

Chaverri P, Liu M, Hodge KT. 2008. A monograph of entomopathogenic genera *Hypocrella*, *Moelleriella* and *Samuelsia* gen nov. (*Ascomycota, Hypocreales, Clavicipitaceae*), and their anamorphs in the Neotropics. Studies in Mycology 60: 1–66. http://dx.doi.org/10.3114/sim.2008.60.01

Gams W, Humber RA, Jaklitsch W, Kirschner R, Stadler M. 2012. Minimizing the chaos following the loss of Article 59: Suggestions for a discussion. Mycotaxon 119: 495–507. http://dx.doi.org/10.5248/119.495

Hall TA, 1999. BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. Nucleic Acids Symposium Series 41: 95–98. http://www.mbio. ncsu.edu/bioedit/bioedit.html [Accessed 13 Feb 2005].

Hawksworth DL, Crous PW, Redhead SA, Reynolds DR, Samson RA, Seifert KA, Taylor JW, Wingfield MJ, et al. 2011. The Amsterdam declaration on fungal nomenclature. IMA Fungus 2: 105–112. http://dx.doi.org/10.5598/imafungus.2011.02.01.14

Heritage J, Evans EGV, Killington RA. 1996. Introductory microbiology. Cambridge University Press. Cambridge. http://dx.doi.org/10.1017/CBO9781139170635

Hywel-Jones NL, Evans HC. 1993. Taxonomy and ecology of *Hypocrella discoidea* and its anamorph *Aschersonia samoensis*. Mycological Research 97: 871–876. http://dx.doi.org/10.1016/S0953-7562(09)81165-9

Kornerup A, Wanscher JH. 1962. Reinhold color atlas. Reinhold Publishing Corporation, New York. 224 p.

Mains EB. 1959a. North American species of *Aschersonia* parasitic on *Aleyrodidae*. J. Insect Pathol. 1: 43–47.

Mains EB. 1959b. Species of *Aschersonia* (*Sphaeropsidales*). Lloydia 22(3): 215–221.

McNeill J, Barrie FR, Buck WR, Demoulin V, Greuter W, Hawksworth DL, Herendeen PS, Knapp S, Marhold K, Prado J, Prud'homme van Reine WF, Smith GE, Wiersema JH, Turland NJ (eds), 2012. International Code of Nomenclature for algae, fungi, and plants (Melbourne Code) adopted by the Eighteenth International Botanical Congress Melbourne, Australia, July 2011 (Regnum Vegetabile No. 154). Koeltz Scientific Books, Königstein.

Mongkolsamrit S, Luangsa-ard JJ, Spatafora JW, Sung GH, Hywel-Jones NL. 2009. A combined ITS rDNA and beta-tubulin phylogeny of Thai species of *Hypocrella* with non-fragmenting ascospores. Mycological Research 113: 684–699. http://dx.doi.org/10.1016/j.mycres.2009.02.004

Mongkolsamrit S, Nguyen TT, Tran NL, Luangsa-ard JJ. 2011. *Moelleriella pumatensis*, a new entomogenous species from Vietnam. Mycotaxon 117: 45–51. http//dx.doi.org/10.5248/117.45

Montagne JPFC. 1848. Sixième centurie de plantes cellulaires exotiques nouvelles. Cryptogamae Taitenses. Annales des Sciences Naturelles Botanique, Série 3, 10: 106–136.

Petch T. 1921. Studies in entomogenous fungi. II. The genera of *Hypocrella* and *Aschersonia*. Annals of the Royal Botanic Gardens Peradeniya 7: 167–278.

Qiu JZ, Guan X. 2010. A new species of *Aschersonia* (*Clavicipitaceae, Hypocreales*) from China. Mycotaxon 111: 471–475. http://dx.doi.org/10.5248/111.471

Qiu JZ, Ma HF, Wang YY, Guan X. 2009. Two *Aschersonia* species from Fujian new to China. Mycosystema 28(1): 60–63.

Qiu JZ, Sun CY, Guan X. 2010. A new pathogen of scale insects, *Aschersonia fusispora* sp. nov. (*Clavicipitaceae*) from Guangxi Province, China. Mycotaxon 113: 81–85. http://dx.doi.org/10.5248/113.81

Rehner SA. 2001. Primers for Elongation Factor 1-alpha (EF1-alpha). Available from: http://ocid.nacse.org/research/deephyphae/EF1primer.pdf

Sung G-H, Spatafora JW, Zare R, Hodge KT, Gams W. 2001. A revision of *Verticillium* sect. *Prostrata* II. Phylogenetic analyses of SSU and LSU nuclear rDNA sequences from anamorphs and teleomorphs of the *Clavicipitaceae*. Nova Hedwigia 72: 311–328.

Taylor JE. 2011. One fungus = one name: DNA and fungal nomenclature twenty years after PCR. IMA Fungus 2: 113–120. http://dx.doi.org/10.5598/imafungus.2011.02.02.01

Vilgalys R, Sun BL. 1994. Ancient and recent patterns of geographic speciation in the oyster mushroom *Pleurotus* revealed by phylogenetic analysis of ribosomal DNA sequences. Proc Natl Acad Sci USA 91: 4599–4603. http://dx.doi.org/10.1073/pnas.91.10.4599

ISSN (print) 0093-4666 © 2014. Mycotaxon, Ltd. ISSN (online) 2154-8889

MYCOTAXON

http://dx.doi.org/10.5248/129.41

Volume 129(1), pp. 41–46 *July–September 2014*

South Florida microfungi: *Linkosia longirostrata*, a new hyphomycete on paurotis palm

Gregorio Delgado

EMLab P&K North Phoenix, 1501 West Knudsen Drive, Phoenix, AZ 85027, U.S.A.

* Correspondence to: *gdelgado@emlabpk.com*

Abstract — *Linkosia longirostrata* sp. nov. is described and illustrated from rachides of dead leaves of *Acoelorrhaphe wrightii* collected in southeastern Florida, U.S.A. The fungus is distinct in having no or very reduced 1–2-septate conidiophores, smooth or slightly verrucose determinate or occasionally percurrent conidiogenous cells, and narrowly obclavate to long obclavate rostrate finely roughened 9–18-distoseptate conidia with 0–2 dark brown constrictions and a long slender straight rostrum with 0–5 intercalary nodular swellings. Differences and similarities with morphologically similar *Linkosia* species are discussed.

Key words — anamorphic *Ascomycota*, palm fungi, *Sporidesmium*, taxonomy

Introduction

While examining collections of dead plant debris from south Florida, several interesting *Sporidesmium*-like taxa were found colonizing decaying parts of native or introduced palm trees. One clearly fits within *Linkosia* A. Hern.-Gut. & B. Sutton (Hernández-Gutiérrez & Sutton 1997, Wu & Zhuang 2005) in having distoseptate conidia and very reduced or absent conidiophores. After a detailed comparison with previously described *Linkosia* species, the fungus was found to differ in several morphological features and therefore is described here as new.

Materials & methods

Samples of dead leaves of *Acoelorrhaphe wrightii* (Griseb. & H. Wendl.) H. Wendl. ex Becc. (paurotis palm; *Arecaceae*) were collected from a forested area in central Broward County, Florida, U.S.A., in 2010. The samples were cut into small pieces and placed in plastic bags for later processing and examination according to Cannon & Sutton (2004). Fungal structures were mounted in lacto-cotton blue and 100 measurements were made at 1000× magnification whenever possible. Minimum, maximum, 5th and 95th percentiles were calculated for all measurements using Microsoft Excel 2007, with

extreme values given in parentheses when different from percentiles. Microphotographs were taken using an Olympus BX-45 microscope and edited using Adobe Photoshop. The type specimen and other specimen including semi-permanent slides are deposited in the Herbarium of the U.S. National Fungus Collections (BPI).

Taxonomy

Linkosia longirostrata G. Delgado, **sp. nov.** PLATE 1

MYCOBANK MB809951

Differs from *Linkosia coccothrinacis* in having very reduced 1–2-septate conidiophores, occasionally percurrent conidiogenous cells, and larger finely roughened long obclavate-rostrate conidia with more distosepta and a rostrum with 0–5 intercalary nodular swellings.

TYPE — U.S.A. Florida, Broward Co., Plantation, Plantation Heritage Park, Anne Kolb Memorial Trail, 26°06′25″N 80°13′19″W, on rachides of dead leaves of *Acoelorrhaphe wrightii*, 30.V.2010, coll. G. Delgado (**Holotype**: BPI 884152H).

ETYMOLOGY — *longirostrata*, from the Latin *longus* (long) and *rostratus* (beaked), referring to the long conidial rostrum

COLONIES effuse, hairy, brown. MYCELIUM superficial, composed of branched, septate, pale brown to brown hyphae, 2–3 µm wide. CONIDIOPHORES absent or very reduced, erect, straight, 1–2-septate, dark brown, often with a lobed base, 19–30(–37) µm long, 7–15 µm wide at base. CONIDIOGENOUS CELLS monoblastic, integrated, terminal, determinate or occasionally percurrent, solitary, simple, subcylindrical or lageniform, smooth or slightly verrucose, tapering toward the truncate apex, brown to dark brown, 13–22 × 6–11 µm, 3.5–5 µm wide at the apex, with 0–1 ampulliform, brown, rarely 1-septate proliferation. CONIDIAL SECESSION schizolytic. CONIDIA holoblastic, straight or slightly curved, narrowly obclavate to long obclavate rostrate, 9–18-distoseptate with 0–4 pigmented distosepta, pale brown, darker toward the apex, sometimes with 0–2 dark brown constrictions, finely rough, (73–)91–158(–172) µm long (including rostrum), 6.5–12 µm wide; basal cell cylindrical or conico-truncate, dark brown to blackish brown, 6–11 × 3.5–5 µm; rostrum slender, straight, pale brown to brown, subhyaline at the tip, ≤82 µm long, with 0–5 intercalary, nodular swellings, 3–5 µm wide. TELEOMORPH unknown.

ADDITIONAL SPECIMEN EXAMINED — U.S.A. Florida, Broward Co., Plantation, Plantation Heritage Park, Anne Kolb Memorial Trail, 26°06′25″N 80°13′19″W, on rachides of dead leaves of *Acoelorrhaphe wrightii*, 30.V.2010, coll. G. Delgado (BPI 884154H).

Discussion

Hernández-Gutiérrez & Sutton (1997) introduced *Linkosia* in the context of a morphology-based reassessment of *Sporidesmium* Link that emphasized conidial septation, conidiophore presence or absence, and conidiophore proliferation (Subramanian 1992). The type species, *Sporidesmium coccothrinacis*

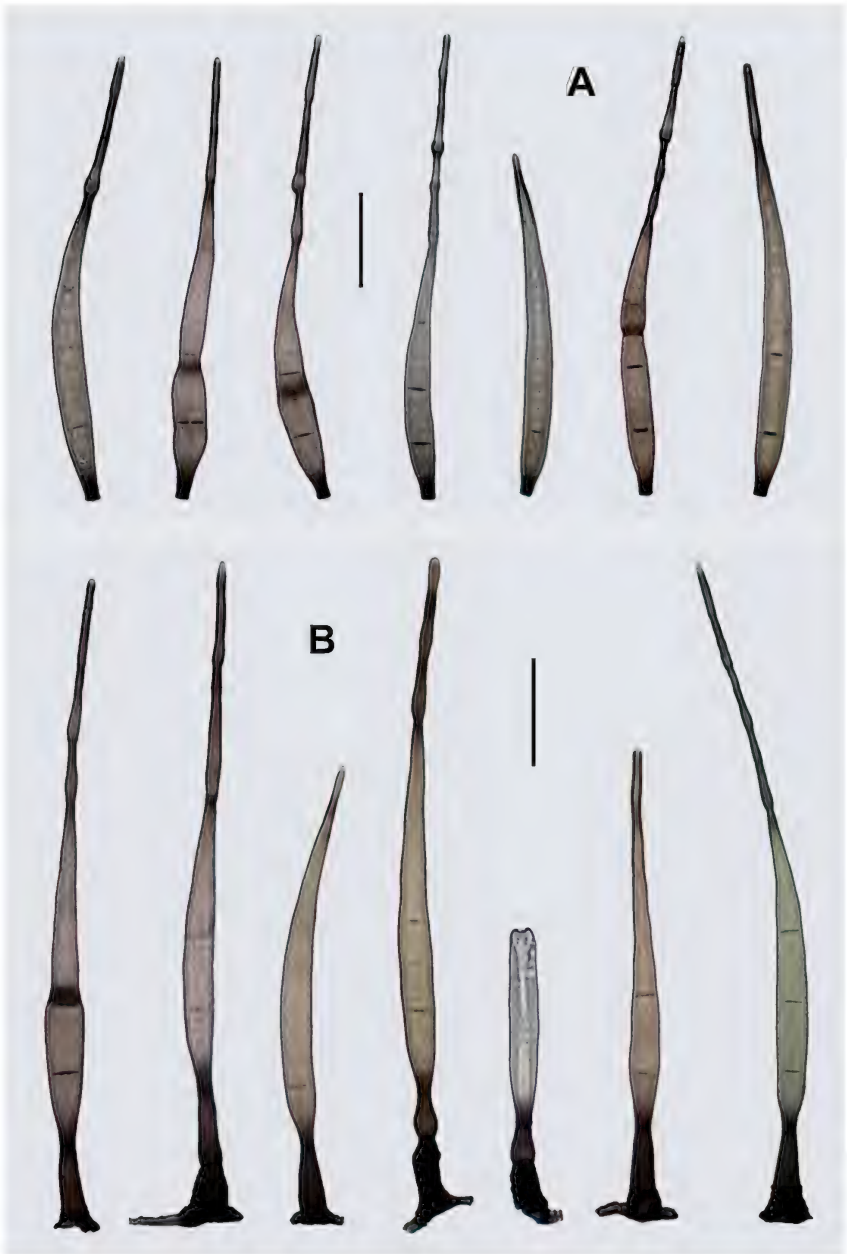


Plate 1. *Linkosia longirostrata* (holotype, BPI 884152H): A. Conidia. B. Conidiophores, conidiogenous cells with or without proliferations, and mature and immature conidia. Scale bars = 30 µm.

A. Hern.-Gut. & J. Mena (≡ *Linkosia coccothrinacis;* Hernández-Gutiérrez & Sutton 1997), is a peculiar hyphomycete characterized by conidiophores reduced to a single monoblastic conidiogenous cell and distoseptate conidia (Hernández-Gutiérrez & Mena 1994). Subsequently, nine additional species have been described or transferred to the genus based on differences in conidial morphology including shape, number of distosepta, dimensions, wall texture and presence or absence of apical appendages (Almeida et al. 2014, Castañeda et al. 2000, Ma et al. 2011, Wu & Zhuang 2005, Zhang et al. 2009). Multigene-sequence data indicate that *Linkosia*, as well as other morphologically circumscribed *Sporidesmium*-like genera, are polyphyletic (Shenoy et al. 2007, Iturriaga et al. 2008). The few phylogenetic studies conducted on this generic complex have revealed the diverse or uncertain affinities of *Linkosia* species within the *Sordariomycetes* (Shenoy et al. 2006, Yang et al. 2010). The genus needs to be redefined in the light of molecular data but very few sequences are currently available in GenBank and one (attributed to *L. fusiformis* W.P. Wu) is apparently a contaminant (Summerbell et al. 2011). For that reason and in the absence of a culture isolate and molecular data, the present fungus is placed in *Linkosia* for diagnostic purposes, following the traditional morphological approach.

Linkosia longirostrata is morphologically unique among *Linkosia* species. Conidiogenous cells, which are slightly verrucose or smooth, are usually determinate but sometimes proliferate percurrently at least once to form an ampulliform proliferation at the apex which can be either 0- or 1-septate, a feature not previously seen in any other *Linkosia* species but present in the morphologically similar genus *Stanjehughesia* Subram. (Castañeda & Kendrick 1990, McKenzie 1995). The conidia are narrowly obclavate but mostly long obclavate rostrate with a long slender straight rostrum where up to 5 nodular swellings form at intervals along its length. These swellings apparently relate to different stages of rostrum elongation. The conidial tip, usually tapering to 1.5–2 µm, widens to 3–5 µm diam. and is at first rounded, after which the rostrum elongates to a certain length, gradually tapers, darkens, and widens again at a further point to form a new swelling, giving the rostrum a knotty appearance. One or two constrictions with a distinct dark brown band are often present at some distosepta, and sometimes the conidium between the constriction and basal cell turns dark brown as well. Also a few distosepta (up to four in certain conidia) appear pigmented and darker than the others. Younger conidia are smooth, but older ones are finely roughened with basal cells that are cylindrical or conico-truncate and distinctly dark brown to blackish brown. *Linkosia coccothrinacis* (A. Hern.-Gut. & J. Mena) A. Hern.-Gut. & B. Sutton (Hernández-Gutiérrez & Sutton 1997), a saprobe on dead leaves of a palm tree found in Cuba, is morphologically similar, sharing dark brown constrictions, a

few pigmented distosepta, and conico-truncate darker basal cells. Its conidia, however, are smaller (43.7–71.5 × 7–10.6 µm) and smooth and have only 4–7 distosepta and a short rostrum without swellings.

Additionally, conidiophores in *L. longirostrata* are absent or present, and when present they are very reduced, usually 1- but occasionally 2-septate and often with a lobed base. Three *Linkosia* species were originally described as producing short 0–2-septate conidiophores: *L. ponapensis* (Matsush.) R.F. Castañeda et al. (Matsushima 1981, as *Sporidesmium ponapense*), *L. refugia* (B. Sutton & Pascoe) D.A.C. Almeida & Gusmão, and *L. canescens* (B. Sutton & Pascoe) D.A.C. Almeida & Gusmão (Sutton & Pascoe 1988, as *Janetia refugia* and *J. canescens*), while the remaining species have only lageniform or ampulliform conidiogenous cells that form directly on the superficial mycelium (Santa Izabel et al. 2013, Wu & Zhuang 2005). *Linkosia ponapensis* is clearly separated from *L. longirostrata* by its cylindrical conidiogenous cells and shorter (34–50(–70) µm) naviculiform conidia with (3–)5–7(–9) distosepta and an apical 6–25 µm long subulate appendage. *Linkosia refugia* is distinguished from *L. longirostrata* by its very rarely branched conidiophores and smaller (31–37 × 7–8 µm) obpyriform 4–6-distoseptate conidia that gradually taper towards an obtuse paler apex but are not distinctly rostrate. In *L. canescens*, conidiogenous cells are mono- or polyblastic, bearing a single or 2–3 denticulate conidiogenous loci per cell; therefore, despite the presence of distoseptate conidia, this fungus is not considered congeneric with *Linkosia* as presently circumscribed and is better retained in *Janetia* M.B. Ellis (Ellis 1976, Goh & Hyde 1996).

Acknowledgments

I am grateful to Drs. A. Hernández-Gutiérrez (Universidade Federal do Para) and De-Wei Li (Connecticut Agricultural Experiment Station) for serving as presubmission reviewers and their many helpful comments on the manuscript. Thanks are also due to Dominick Shannon (USDA) for depositing specimens in BPI and Elsa Delgado for assistance in the field. Joshua Cox and Dr. Kamash Ramanathan (EMLab P&K) are gratefully acknowledged for provision of laboratory facilities and financial support.

Literature cited

Almeida DAC, Miller AN, Gusmão LFP. 2014. New species and combinations of conidial fungi from the semi-arid Caatinga biome of Brazil. Nova Hedwigia 98: 431–447. http://dx.doi.org/10.1127/0029-5035/2013/0162

Cannon P, Sutton B. 2004. Microfungi on wood and plant debris. 217–239, in: G Mueller et al. (eds). Biodiversity of fungi: inventory and monitoring methods. Elsevier Academic Press, Burlington.

Castañeda RF, Kendrick B. 1990. Conidial fungi from Cuba: II. University of Waterloo Biol. Ser. 33. 61 p.

Castañeda RF, Decock C, Saikawa M, Gené J, Guarro J. 2000. *Polyschema obclaviformis* sp. nov., and some new records of hyphomycetes from Cuba. Cryptog. Mycol. 21: 215–220. http://dx.doi.org/10.1016/S0181-1584(00)01051-4

Ellis MB. 1976. More dematiaceous hyphomycetes. Commonwealth Mycological Institute, Kew.

Goh TK, Hyde KD. 1996. *Janetia curviapicis*, a new species, and an emended description of the genus. Mycologia 88: 1014–1021. http://dx.doi.org/10.2307/3761066

Hernández-Gutiérrez A, Mena J. 1994. *Sporidesmium coccothrinacis* Hernández & Mena, sp. nov. Bol. Soc. Micol. Madrid 19: 313–314.

Hernández-Gutiérrez A, Sutton BC. 1997. *Imimyces* and *Linkosia*, two new genera segregated from *Sporidesmium sensu lato*, and redescription of *Polydesmus*. Mycol. Res. 101: 201–209. http://dx.doi.org/10.1017/S0953756296002419

Iturriaga T, Hawksworth DL, Crane JL. 2008. '*Sporidesmium lichenicola*' sp. nov., a new lichenicolous fungus on *Leptogium* from Venezuela. Mycologia 100: 392–396. http://dx.doi.org/10.3852/06-166R

Ma J, Ma LG, Zhang YD, Zhang XG. 2011. Three new hyphomycetes from southern China. Mycotaxon 117: 247–253. http://dx.doi.org/10.5248/117.247

Matsushima T. 1981. Matsushima mycological memoirs no. 2. Published by the author, Kobe.

McKenzie EHC. 1995. Dematiaceous hyphomycetes on *Pandanaceae*. 5. *Sporidesmium* sensu lato. Mycotaxon 56: 9–29.

Santa Izabel TS, Cruz ACR, Gusmão LFP. 2013. Conidial fungi from the semi-arid Caatinga biome of Brazil. *Ellisembiopsis* gen. nov., new variety of *Sporidesmiella* and some notes on *Sporidesmium* complex. Mycosphere 4: 156–163. http://dx.doi.org/10.5943/mycosphere/4/2/1

Shenoy BD, Jeewon R, Wu WP, Bhat DJ, Hyde KD. 2006. Ribosomal and RPB2 DNA sequence analyses suggest that *Sporidesmium* and morphologically similar genera are polyphyletic. Mycol. Res. 110: 916–928. http://dx.doi.org/10.1016/j.mycres.2006.06.004

Shenoy BD, Jeewon R, Hyde KD. 2007. Impact of DNA sequence-data on the taxonomy of anamorphic fungi. Fungal Diversity 26: 1–54.

Subramanian CV. 1992. A reassessment of *Sporidesmium* (hyphomycetes) and some related taxa. Proc. Nat. Acad. Sci. India B58: 179–190.

Summerbell RC, Gueidan C, Schroers HJ, de Hoog GS, Starink M, Arocha Rosete Y, Guarro J, Scott JA. 2011. *Acremonium* phylogenetic overview and revision of *Gliomastix*, *Sarocladium*, and *Trichothecium*. Stud. Mycol. 68: 139–162. http://dx.doi.org/10.3114/sim.2011.68.06

Sutton BC, Pascoe IG. 1988. Some dematiaceous hyphomycetes from branches and phyllodes of *Acacia* in Australia. Aust. Syst. Bot. 1: 127–138. http://dx.doi.org/10.1071/SB9880127

Wu WP, Zhuang W. 2005. *Sporidesmium*, *Endophragmiella* and related genera from China. Fungal Diversity Press, Hong Kong.

Yang HL, Sun GY, Batzer JC, Crous PW, Groenewald JZ, Gleason ML. 2010. Novel fungal genera and species associated with the sooty blotch and flyspeck complex on apple in China and the USA. Persoonia 24: 29–37. http://dx.doi.org/10.3767/003158510X492101

Zhang K, Ma LG, Zhang XG. 2009. A new hyphomycete species from Guangxi, China. Mycotaxon 108: 123–125. http://dx.doi.org/10.5248/108.123

ISSN (print) 0093-4666 © 2014. Mycotaxon, Ltd. ISSN (online) 2154-8889

MYCOTAXON

http://dx.doi.org/10.5248/129.47

Volume 129(1), pp. 47–56 *July–September 2014*

Taxonomy and phylogeny of *Heterobasidion* in South Korea

Yeongseon Jang[1], Seokyoon Jang[1], Young Woon Lim[2], Changmu Kim[3] & Jae-Jin Kim[1]*

[1]*Division of Environmental Science & Ecological Engineering, College of Life Sciences & Biotechnology, Korea University, 145 Anam-ro, Seongbuk-gu, Seoul, 136-701, Korea*

[2]*School of Biological Sciences, Seoul National University, Seoul, 151-747, Korea*

[3]*National Institute of Biological Resources, Environmental Research Complex, Incheon, 404-708, Korea*

*Correspondence to: *jae-jinkim@korea.ac.kr*

Abstract — ITS and TEF gene sequences from eleven basidiome collections from South Korea and one mycelial culture were analyzed to infer the phylogeny of Korean *Heterobasidion* species. Comparison of morphological characters with phylogenetic analyses revealed that specimens previously recorded as '*H. annosum*' and '*H. araucariae*' in fact represent *H. ecrustosum*, while those recorded as '*H. insulare*' represent *H. orientale*. The two species currently recognized in South Korea are described and illustrated.

Key words — *Basidiomycota, Bondarzewiaceae*, polypore

Introduction

Heterobasidion Bref. is a well-known polypore genus that includes the destructive tree pathogen, *H. annosum* (Fr.) Bref. The genus is characterized by resupinate to pileate basidiocarps, a dimitic hyphal system with mostly simple septate generative hyphae and dextrinoid skeletal hyphae, and finely asperulate inamyloid basidiospores (Gilbertson & Ryvarden 1986). In addition, its skeletal hyphae are cyanophilous in Cotton Blue (Dai & Korhonen 2009). Until recently, five species — *H. annosum*, *H. araucariae* P.K. Buchanan, *H. insulare* (Murrill) Ryvarden, *H. pahangense* Corner, and *H. rutilantiforme* (Murrill) Stalpers — were accepted, but both *H. annosum* and *H. insulare* have been found to represent species complexes (Korhonen 1978, Dai et al. 2002), so that now eleven *Heterobasidion* species are recognized (Dai & Korhonen 1999,

Tokuda et al. 2009, Dai & Korhonen 2009, Otrosina & Garbelotto 2010). Dai et al. (2003), Tokuda et al. (2009), and Dai & Korhonen (2009) accept *H. australe* Y.C. Dai & Korhonen, *H. ecrustosum*, *H. linzhiense* Y.C. Dai & Korhonen, *H. orientale*, and *H. parviporum* Niemelä & Korhonen in northeast Asia (China and Japan).

In Korea, Lee (1988), Lee & Jung (2006), and Paul et al. (2012) have reported three *Heterobasidion* species: *H. annosum*, *H. araucariae,* and *H. insulare. Heterobasidion insulare* was reported (as *Fomitopsis insularis*) from a conifer trunk (Lee 1988), *H. annosum* was found at the base of *Pinus rigida* (Lee & Jung 2006), and *H. araucariae* was found as an endophyte from roots of red pepper, *Capsicum annuum* (Paul et al. 2012). However, as none of these three species had previously been reported for northeast Asia, re-examination of those materials was required.

In this study, we list the *Heterobasidion* species in South Korea and describe the morphological characteristics for specimens collected in the country and, when possible, for voucher specimens from previous studies. In addition, we analyzed sequences from the internal transcribed spacer rDNA (ITS) and translation elongation factor 1-alpha (TEF). The ITS was selected because it is used for barcoding fungal species (Schoch et al. 2012), and the TEF has been used to infer species relationships within *Heterobasidion* (Ota et al. 2006, Paul et al. 2012). We describe and illustrate the two accepted species and compare their key characters with the *Heterobasidion* species in northeast Asia.

Materials & methods

Collection and morphological examination

The basidiocarps of *Heterobasidion* spp. identified by Lee & Jung (2006) and materials deposited in Seoul National University Fungal Collection (SFC) and the Korea University Culture Collection (KUC) were used in this study (Table 1). The macro- and microscopic characteristics of the specimens were examined according to Jang et al. (2013). Color codes in the descriptions follow Munsell (2000).

Phylogenetic analysis

Genomic DNAs were extracted according to Jang et al. (2013) from the materials in Table 1. PCR reactions for ITS regions were performed using Accupower PCR Premix Kit (Bioneer, Korea) with the primers ITS1F/ITS4 according to Jang et al. (2013). TEF region was amplified using the same kit with the primers, EF1-728F (5′-CATCGAGAAGTTCGAGAAGG-3′) /EF1-1567R (5′-ACHGTRCCRATACCACC-RATCTT-3′) and the PCR conditions: an initial denaturation step of 95°C for 7 min, followed by 35 cycles of 95°C for 30 s, 58°C for 30 s, and 72°C for 1 min, with an elongation step of 72°C for 7 min at the end. The DNA was sequenced by the Macrogen sequencing service (Seoul, Korea). Sequences obtained in this study were deposited in GenBank, NCBI; the accession numbers are shown in Table 1. The ITS sequences

Table 1. The Korean *Heterobasidion* specimens and their ITS and TEF sequences used in this study.

Species	Original determination	Specimen no.[a]	GenBank ITS No.	GenBank TEF No.
H. ecrustosum	*H. annosum*	KUC20080904-43	—	—
	H. annosum	KUC20110916-44	KF218829	—
	H. annosum	KUC20111001-14	KF218830	KF154267
	H. annosum	KUC20120810-11	KF218831	KF154268
	H. annosum	KUC20120810-14	KF218832	KF154269
	H. araucariae	CNU081069	JQ691621[b]	JQ691622[b]
	H. annosum	SFC 20020927-11	—	—
	H. annosum	SFC 20120820-01	KF218834	KF154270
H. orientale	*H. insulare*	KUC20081030C-04	KF218828	—
	H. insulare	KUC20121019-01	—	KF154271
	H. insulare	KUC20121019-30	KF218833	KF154272
	H. insulare	KUC20121123-14	—	KF154273

[a] Examined basidiocarps now deposited in National Institute of Biological Resources (KB); *H. ecrustosum* CNU081069 is a mycelial isolate from Paul et al (2012).

[b] Sequences retrieved from GenBank, NCBI.

were aligned with the *Heterobasidion* sequences from Dalman et al. (2010). TEF sequences were aligned with the sequences from Ota et al. (2006) and Paul et al. (2012). The sequence alignment, model selection, and Bayesian phylogenetic analyses were performed according to Jang et al. (2013).

Results & discussion

Morphological comparisons

Although the voucher specimens of '*Heterobasidion insulare*' in Lee (1988) were not available, we feel that his fungus represents *H. orientale* based on his description and colored illustration of the basidiocarps. He described the basidiocarps as sessile and 2.5–5 × 4–8 × 1–1.5 cm; the pileus had a brick-red surface and white to cream margin. The 1.5–3 mm context was white to cream, the tubes were 1 cm with round to labyrinthiform pores, and the spores were subglobose and 4–5 µm long. In contrast, *H. insulare* has a mostly light brown pileus surface and round to angular (not labyrinthiform) pores (Tokuda et al. 2009). In addition, other specimens formerly known as '*H. insulare*' (Table 1) are actually *H. orientale:* Tokuda et al. (2009) note that specimens previously recognized as *H. insulare* in China and Japan represent *H. orientale* and that *H. insulare* is known only from the type specimen found in the Philippines.

Re-examination '*Heterobasidion annosum*' specimen SFC 20020927-11 from Lee & Jung (2006) revealed that it represents instead *H. ecrustosum,*

and the species description in Lee & Jung (2006) is quite similar to that of *H. ecrustosum* by Tokuda et al. (2009). Furthermore, all specimens previously identified as '*H. annosum*' have been since identified as *H. ecrustosum* (Table 1). Dai et al. (2003) showed that *H. annosum* has not been found from eastern Asia and Altai region and that Russia represents its easternmost distribution.

'*Heterobasidion araucariae*' from Paul et al. (2012) was found as an endophyte. The cultural characteristics of *H. araucariae* and *H. ecrustosum* are similar. The data of Paul et al. (2012) for their '*H. araucariae*' (conidiophores <300 × 5.5–10.5 µm; conidia 4–11.5 × 2–7 µm) lie within the data range of Tokuda et al. (2009) for *H. ecrustosum* (conidiophores <300 × 4–10 µm; conidia 3.2–17.1 × 2.9–12 µm), but only partially overlap the data range of Buchanan (1988) for authentic *H. araucariae* (conidiophores <400 × 5.5–13.5 µm; conidia 5–16 × 3–13.5 µm). Authentic *H. araucariae* has been found only in Australia, New Zealand, and Fiji (Buchanan 1988; Ota et al. 2006; Tokuda et al. 2009), and Tokuda et al. (2009) consider it a species restricted to the southern hemisphere.

Phylogeny

Among the 11 specimens examined in this study, ITS gene region sequences were obtained from seven specimens (594–627 bp) and TEF gene region sequences from seven specimens (718–774 bp) (Table 1). No sequence was amplified from KUC20080904-43 and SFC 20020927-11. The phylogenetic analyses supported our morphological conclusions that the examined '*H. annosum*' and '*H. araucariae*' specimens represent *H. ecrustosum* and that the examined '*H. insulare*' specimens represent *H. orientale*.

Bayesian analysis of ITS sequences (not presented) was congruent with the tree from Dalman et al. (2010). No intraspecific ITS sequence variation was found in Korean *H. ecrustosum* and *H. orientale*. *Heterobasidion ecrustosum* was monophyletic with *H. araucariae* and our *H. ecrustosum* ITS sequences were 99% similar (5 positions different) to *H. araucariae* from Australia and New Zealand (GenBank FJ627521, FJ627522, FJ627526, and FJ627527). *Heterobasidion orientale* was monophyletic and sister to the *H. araucariae*/*H. ecrustosum* clade.

The TEF gene region tree (Fig. 1) showed a topology similar to that of Ota et al. (2006) except that Japanese isolates of *H. parviporum* did not form a separate clade but were positioned on the basal part of the clade comprising *H. occidentale*, *H. abietinum*, and *H. parviporum*. Korean *H. orientale* collections produced two different TEF sequences although they were collected from the same location; their basal position within the *H. orientale* clade was supported with high posterior probability (1.0 p.p.). *Heterobasidion orientale*

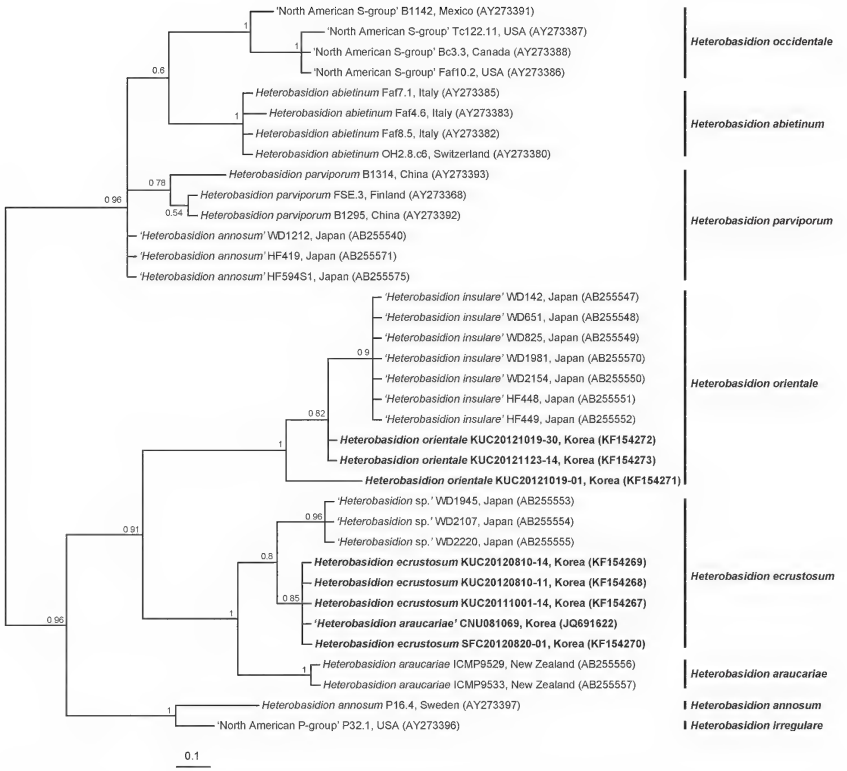

'North American S-group' B1142, Mexico (AY273391)
'North American S-group' Tc122.11, USA (AY273387)
'North American S-group' Bc3.3, Canada (AY273388)
'North American S-group' Faf10.2, USA (AY273386)
Heterobasidion occidentale
Heterobasidion abietinum Faf7.1, Italy (AY273385)
Heterobasidion abietinum Faf4.6, Italy (AY273383)
Heterobasidion abietinum Faf8.5, Italy (AY273382)
Heterobasidion abietinum OH2.8.c6, Switzerland (AY273380)
Heterobasidion abietinum
Heterobasidion parviporum B1314, China (AY273393)
Heterobasidion parviporum FSE.3, Finland (AY273368)
Heterobasidion parviporum B1295, China (AY273392)
'Heterobasidion annosum' WD1212, Japan (AB255540)
'Heterobasidion annosum' HF419, Japan (AB255571)
'Heterobasidion annosum' HF594S1, Japan (AB255575)
Heterobasidion parviporum
'Heterobasidion insulare' WD142, Japan (AB255547)
'Heterobasidion insulare' WD651, Japan (AB255548)
'Heterobasidion insulare' WD825, Japan (AB255549)
'Heterobasidion insulare' WD1981, Japan (AB255570)
'Heterobasidion insulare' WD2154, Japan (AB255550)
'Heterobasidion insulare' HF448, Japan (AB255551)
'Heterobasidion insulare' HF449, Japan (AB255552)
Heterobasidion orientale KUC20121019-30, Korea (KF154272)
Heterobasidion orientale KUC20121123-14, Korea (KF154273)
Heterobasidion orientale KUC20121019-01, Korea (KF154271)
Heterobasidion orientale
'Heterobasidion sp.' WD1945, Japan (AB255553)
'Heterobasidion sp.' WD2107, Japan (AB255554)
'Heterobasidion sp.' WD2220, Japan (AB255555)
Heterobasidion ecrustosum KUC20120810-14, Korea (KF154269)
Heterobasidion ecrustosum KUC20120810-11, Korea (KF154268)
Heterobasidion ecrustosum KUC20111001-14, Korea (KF154267)
'Heterobasidion araucariae' CNU081069, Korea (JQ691622)
Heterobasidion ecrustosum SFC20120820-01, Korea (KF154270)
Heterobasidion ecrustosum
Heterobasidion araucariae ICMP9529, New Zealand (AB255556)
Heterobasidion araucariae ICMP9533, New Zealand (AB255557)
Heterobasidion araucariae
Heterobasidion annosum P16.4, Sweden (AY273397)
Heterobasidion annosum
'North American P-group' P32.1, USA (AY273396)
Heterobasidion irregulare
0.1

FIG. 1. 50% major-rule consensus tree of Korean *Heterobasidion* spp. and allies using TEF region sequences. The tree, containing 36 taxa and 258 characters, was constructed from 15,000 trees produced by Bayesian analysis and was mid-point rooted. Posterior probabilities ≥50% are given. Korean specimens are shown in bold type. For each node, the former names, isolate numbers, and localities are given, and the species names accepted by recent studies (Tokuda et al. 2009, Otrosina & Garbelotto 2010) are presented on the right side of the tree. GenBank accession numbers are provided in parentheses.

KUC20121019-30 and KUC20121123-14 were 98% similar to Japanese isolates (254 out of 258 sequences matched), and KUC20121019-01 was 96% similar (247 out of 258 sequences matched). Korean *H. ecrustosum* collections (including '*H. araucariae*' CNU081069 from Paul et al. 2012) had 100% identical TEF sequences and were sister to Japanese *H. ecrustosum* isolates with moderate support (0.8 p.p.). The *H. ecrustosum* clade again clustered with authentic *H. araucariae* with high support (1.0 p.p.). Korean *H. ecrustosum* collections were 98% similar to Japanese *H. ecrustosum* (245 out of 251 sequences matched) and 97% similar to *H. araucariae* (242 out of 249 sequences matched).

Taxonomy

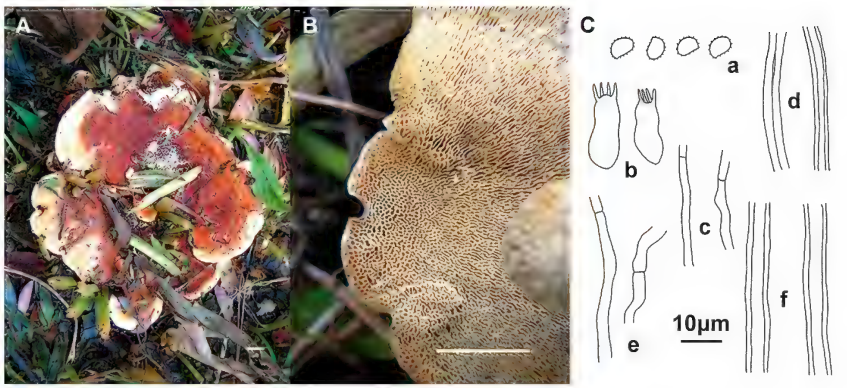


Fig. 2. *Heterobasidion orientale* (KUC20121019-01). A, B. Basidiocarp. Scale bar = 1 cm. C. Microscopic features. a, basidiospores; b, basidia; c, generative hyphae from trama; d, skeletal hyphae from trama; e, generative hyphae from subiculum; f, skeletal hyphae from subiculum.

Heterobasidion orientale Tokuda, T. Hatt. & Y.C. Dai,
Mycoscience 50: 193. 2009. Fig. 2

Basidiocarps annual, sessile, solitary to imbricate, ≤7 × 5 × 2 cm. Pileus broadly attached to the substrate, dimidiate, applanate to elongated. Pileus surface glabrous, sometimes radially rugose when dry, zonate with dusky red (10R3/3) to dark red (7.5R3/6) in the middle, yellow (10YR7/6) to very pale brown (10YR8/2-3) near the margin. Pileus margin acute. Pore surface very pale brown (10YR8/4) to yellow (10YR7/6-8, 10YR8/6-8). Pores round to angular or labyrinthiform, 2–4/mm, dissepiments eroded. Sterile margin 0.5 mm wide or absent. Context corky, very pale brown (10YR8/2), 1–2 mm thick. Tubes 3–6 mm deep, concolorous with context.

Hyphal system dimitic; generative hyphae without clamp connection and skeletal hyphae.

Context generative hyphae without clamp connections, hyaline, thin to slightly thick-walled, IKI–, 2.0–3.5 µm in diameter; skeletal hyphae abundant, hyaline, thick-walled, dextrinoid, 4.5–6.5 µm in diameter.

Tubes generative hyphae without clamp connections, hyaline, thin-walled, IKI–, 2–2.5 µm in diameter; skeletal hyphae abundant, hyaline, thick-walled, weakly dextrinoid, 3–5 µm in diameter; cystidia none; basidia barrel-shaped, 4-sterigmate, slightly constricted in the middle, 17–29 × 5–7 µm.

Basidiospores globose to subglobose, hyaline, finely asperulate, IKI–, (4.6–)4.8–6.7 × 3.9–5.4(–5.8) µm, L = 5.6 µm, W = 4.5 µm (n = 88/3).

Distribution — China, Japan, and Korea.

Specimens examined: **KOREA**, **Kyeonggi-do**, Mt. Bori, 37°39′06″N 127°32′02″E, on *Pinus densiflora* Siebold & Zucc., 30 October 2008, Yeongseon Jang (KB, KUC20081030C-04). **Gangwon-do**, Mt. Odae, 37°43′59″N 128°35′23″E, on unknown wood, 19 October 2012, Yeongseon Jang (KB, KUC20121019-01); 37°44′13″N 128°35′16″E, *Abies holophylla* Maxim., 19 October 2012, Yeongseon Jang (KB, KUC20121019-30); 37°44′30″N 128°35′03″E, on unknown wood, 23 November 2012, Yeongseon Jang (KB, KUC20121123-14).

Remarks — The basidia of Korean *H. orientale* specimens are larger than those given by Tokuda et al. (2009).

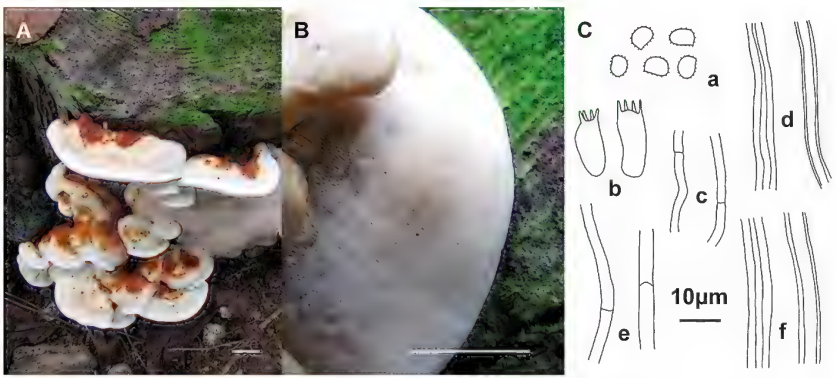


Fig. 3. *Heterobasidion ecrustosum* (KUC20110916-44). A, B. Basidiocarp. Scale bar = 1 cm. C. Microscopic features. a, basidiospores; b, basidia; c, generative hyphae from trama; d, skeletal hyphae from trama; e, generative hyphae from subiculum; f, skeletal hyphae from subiculum.

Heterobasidion ecrustosum Tokuda, T. Hatt. & Y.C. Dai, Mycoscience 50: 196. 2009.
Fig. 3

Basidiocarps annual, sessile, solitary to imbricate, ≤6 × 3.5 × 2 cm. Pileus broadly attached to the substrate, dimidiate, applanate to convex, somewhat inrolled when dry. Pileus surface glabrous, usually rough, subzonate to azonate, radially sulcate in one specimen (KUC20110916-44), very pale brown (10YR8/3-4) to yellow (10YR8/6) or reddish yellow (5YR6/8), with crust at the base or without crust, crust dark yellowish brown (10YR3/4) to very dark brown (10YR2/2) or dark reddish brown (5YR3/4). Pileus margin rounded. Pore surface very pale brown (10YR8/2-4) or pale to light yellowish brown (10YR6/4-6/6), pores round to angular, sometimes elongated, 2–3/mm, dissepiments entire. Sterile margin narrow, concolorous with the pore surface, 0.5–1.5 mm wide, or absent. Context corky, very pale brown (5YR8/2-3), 2–5 mm thick. Tubes concolorous with the context, 1–7 mm deep.

Hyphal system dimitic; generative hyphae without clamp connections and skeletal hyphae.

Context generative hyphae without clamp connections, hyaline, thin to thick-walled, IKI–, 2.5–5 µm in diameter; skeletal hyphae abundant, hyaline, dextrinoid, thick-walled, 3.5–5.5 µm in diameter.

Tubes generative hyphae without clamp connections, hyaline, thin walled, IKI–, 2.0–4.5 µm in diameter; skeletal hyphae abundant, hyaline, dextrinoid, thick-walled, 3–5.5 µm in diameter; cystidia none; basidia barrel-shaped, 4-sterigmate, 17–26 × 4.5–7 µm.

Basidiospores globose to subglobose, hyaline, finely asperulate, IKI–, 4.9–6.9(–7.7) × (3.7–)4.1–5.9(–6.5) µm, L = 5.9 µm, W = 5.0 µm (n = 99/3).

Distribution — China, Japan, and Korea.

Specimen examined: **KOREA**, **Seoul**, Mt. Cheonggye, 37°25′47″N 127°02′51″E, on the branch of wood, 4 September 2008, Yeongseon Jang (KB, KUC20080904-43). Heonilleung, 37°28′03″N 127°05′00″E, on the stump of *Pinus densiflora*, 16 September 2011, Yeongseon Jang (KB, KUC20110916-44). **Jeollabuk-do**, Gochang, 35°27′35″N 126°30′51″E, on unknown wood, 1 October 2011, Yeongseon Jang (KB, KUC20111001-14). **Jeollanam-do**, Wando arboretum, 34°23′05″N 126°39′07″E, on unknown wood, 10 August 2012, Yeongseon Jang (KB, KUC20120810-11); on unknown wood, 10 August 2012, Yeongseon Jang (KB, KUC20120810-14). **Chungcheongbuk-do**, Mt. Songni, on *Pinus rigida* Mill., 27 September 2002, Jin Sung Lee (KB, SFC 20020927-11). **Chungcheongnam-do**, Mt. Gaya, on *Pinus densiflora*, 20 August 2012, Young Woon Lim (KB, SFC 20120820-01).

Remarks — The ranges of basidia and basidiospore sizes of Korean *H. ecrustosum* specimens overlap with those given by Tokuda et al. (2009), but Korean specimens tend to have larger basidia and basidiospores.

We confirm that there are only two *Heterobasidion* species — *H. ecrustosum* and *H. orientale* — in South Korea and compare their characteristics with other northeastern Asian *Heterobasidion* species in Table 2. Because we analyzed

Table 2. Key characteristics of *Heterobasidion* species in northeast Asia.

Species	Basidiocarps	Pores	Basidiospores (µm)	Substrates
H. australe (Dai & Korhonen 2009)	Perennial	Mostly round, occasionally angular, 4–5/mm	4.1–6.2 × 3.3–5.5	*Keteleeria, Picea, Pinus, Pseudolarix, Tsuga*
H. ecrustosum (this study)	Annual	Angular to round, 2–3/mm	4.9–7.7 × 3.7–6.5	*Pinus*
H. linzhiense (Dai et al. 2007)	Annual	Angular, 2–4/mm	5–10 × 4–10	*Abies, Picea*
H. orientale (this study)	Annual	Angular to round or labyrinthiform, 2–4/mm	4.6–6.7 × 3.9–5.8	*Abies, Pinus*
H. parviporum (Tokuda et al. 2009)	Mostly perennial	Round, regular, 4–7/mm	3.3–5.6 × 2.9–4.8	*Abies, Larix, Picea, Pinus, Populus, Tsuga*

only a limited number of specimens, there might be other species present in this region. Thus, further studies are needed to determine the complete diversity of *Heterobasidion* in South Korea.

Acknowledgments

This research was supported by Basic Science Research Program through the National Research Foundation of Korea (NRF) funded by the Ministry of Education (NRF-2013R1A1A2A10011390) and was funded by the project on survey and excavation of Korean indigenous species of NIBR under the Ministry of Environment, Republic of Korea. We are much obliged to Dr. Yu-Cheng Dai and Dr. Hayato Masuya for their valuable suggestions on the manuscript.

Literature cited

Buchanan PK. 1988. A new species of *Heterobasidion* (*Polyporaceae*) from Australasia. Mycotaxon 32: 325–337.

Dai YC, Korhonen K. 2009. *Heterobasidion australe*, a new polypore derived from the *Heterobasidion insulare* complex. Mycoscience 50: 353–356. http://dx.doi.org/10.1007/s10267-009-0491-3

Dai YC, Korhonen K. 1999. *Heterobasidion annosum* group S identified in north-eastern China. European Journal of Forest Pathology 29: 273–279. http://dx.doi.org/10.1046/j.1439-0329.1999.00153.x

Dai YC, Vainio EJ, Hantula J, Niemelä T, Korhonen K. 2002. Sexuality and intersterility within the *Heterobasidion insulare* complex. Mycological Research 106: 1435–1448. http://dx.doi.org/10.1017/S0953756202006950

Dai YC, Vainio EJ, Hantula J, Niemelä T, Korhonen K. 2003. Investigations on *Heterobasidion annosum* s. lat. in central and eastern Asia with the aid of mating tests and DNA fingerprinting. Forest Pathology 33: 269–286. http://dx.doi.org/10.1046/j.1439-0329.2003.00328.x

Dalman K, Olson Å, Stenlid J. 2010. Evolutionary history of the conifer root rot fungus *Heterobasidion annosum* sensu lato. Molecular Ecology 19: 4979–4993. http://dx.doi.org/10.1111/j.1365-294X.2010.04873.x

Gilbertson RL, Ryvarden L. 1986. North American polypores I. Fungiflora, Oslo.

Jang Y, Lee SW, Lim YW, Lee JS, Hattori T, Kim J-J. 2013. The genus *Wrightoporia* in Korea. Mycotaxon 123: 335–341. http://dx.doi.org/10.5248/123.335

Korhonen K. 1978. Intersterility groups of *Heterobasidion annosum.* Communicationes Instituti Forestalis Fenniae 94: 1–25.

Lee JS, Jung HS. 2006. Taxonomic study on Korean Aphyllophorales (5) – on some unrecorded genera and species. Mycobiology 34: 166–175. http://dx.doi.org/10.4489/MYCO.2006.34.4.166

Lee JY. 1988. Coloured Korean mushrooms. Vol. 1. Academy Press. Seoul, Korea.

Munsell Color. 2000. Munsell soil color charts. Gretag Macbeth, New Windsor, NY.

Ota Y, Tokuda S, Buchanan PK, Hattori T. 2006. Phylogenetic relationships of Japanese species of *Heterobasidion—H. annosum* sensu lato and an undetermined *Heterobasidion* sp. Mycologia 98: 717–725. http://dx.doi.org/10.3852/mycologia.98.5.717

Otrosina WJ, Garbelotto M. 2010. *Heterobasidion occidentale* sp. nov. and *Heterobasidion irregulare* nom. nov.: A disposition of North American *Heterobasidion* biological species. Fungal Biology 114: 16–25. http://dx.doi.org/10.1016/j.mycres.2009.09.001

Paul NC, Deng JX, Shin KS, Yu SH. 2012. Molecular and morphological characterization of endophytic *Heterobasidion araucariae* from roots of *Capsicum annuum* L. in Korea. Mycobiology 40: 85–90. http://dx.doi.org/10.5941/MYCO.2012.40.2.85

Schoch CL, Seifert KA, Huhndorf S, Robert V, Spouge JL, Levesque CA, Chen W, Fungal Barcoding Consortium. 2012. Nuclear ribosomal internal transcribed spacer (ITS) region as a universal DNA barcode marker for Fungi. Proceedings of the National Academy of Sciences 109: 6241–6246. http://dx.doi.org/10.1073/pnas.1117018109

Tokuda S, Hattori T, Dai YC, Ota Y, Buchanan PK. 2009. Three species of *Heterobasidion* (*Basidiomycota, Hericiales*), *H. parviporum*, *H. orientale* sp. nov. and *H. ecrustosum* sp. nov. from East Asia. Mycoscience 50: 190–202. http://dx.doi.org/10.1007/s10267-008-0476-7

ISSN (print) 0093-4666 © 2014. Mycotaxon, Ltd. ISSN (online) 2154-8889

MYCOTAXON

http://dx.doi.org/10.5248/129.57

Volume 129(1), pp. 57–61 *July–September 2014*

Two new combinations and a new record of *Zasmidium* from China

Fengyan Zhai[1], W.H. Hsieh[2], Yingjie Liu[1], & Yinglan Guo[3*]

[1] *Henan Institute of Science and Technology, Xinxiang 453003, China*

[2] *Department of Plant Pathology, National Chung Hsing University, Taichung, Taiwan, Republic of China*

[3] *Institute of Microbiology, Chinese Academy of Science, Beijing 100101, China*

* Correspondence to: *guoyl@im.ac.cn*

Abstract — *Stenella gynurae* is recombined as *Zasmidium gynurae*, and *S. bougainvilleae* as *Z. bougainvilleae*; these species are described, illustrated and discussed. *Zasmidium salicis* is also reported as new to China.

Key words —morphology, *Mycosphaerellaceae*, reallocation, taxonomy

Introduction

The type species of *Stenella* Syd., *S. araguata* Syd., which is characterized by pileate condiogenous loci and hila, is placed by molecular sequence analyses in *Teratosphaeriaceae*. In contrast, most other previous *Stenella* species have planate *Cercospora*-like scars and hila and belong in *Mycosphaerellaceae*, where they agree morphologically and cluster phylogenetically with the type of *Zasmidium* Fr.; they have therefore been transferred to *Zasmidium* (Braun et al. 2010a,b). Here we transfer two additional *Stenella* species, *S. gynurae* and *S. bougainvilleae,* which coincide morphologically with the current concept of *Zasmidium*. Another *Zasmidium* species, *Z. salicis*, is reported as new to China.

Taxonomy

Zasmidium gynurae (Sawada & Katsuki) W.H. Hsieh, Y.L. Guo & F.Y. Zhai, **comb. nov.** Fig. 1

MycoBank MB 808069

≡ *Cercospora gynurae* Sawada & Katsuki, Spec. Publ. Coll. Agric., Nat. Taiwan Univ. 8: 218, 1959.

≡ *Stenella gynurae* (Sawada & Katsuki) Goh & W.H. Hsieh, *Cercospora* Similar Fungi Taiwan: 88, 1990.

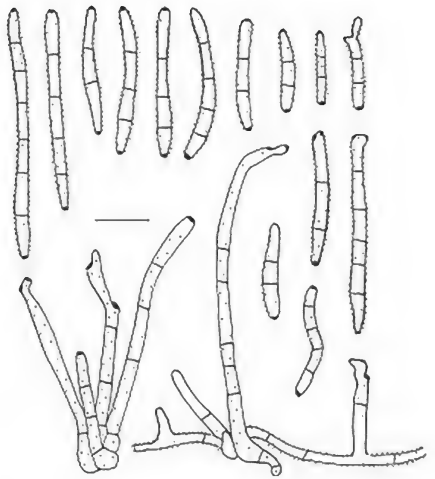


Fig. 1. *Zasmidium gynurae* (NYU-PPE, holotype). Conidiophores and conidia. Scale bar = 10 μm.

Leaf spots circular, greyish, without distinct margin, 3.0–5.0 mm diam. Fruiting hypophyllous. Primary mycelium immersed. Secondary mycelium superficial; hyphae subhyaline to pale olivaceous, septate, branched, finely verruculose, 1.0–2.0 μm diam. Secondary conidiophores arising as lateral branches from external hyphae. Stromata none. Primary conidiophores emerging through stomata, 1–6-fasciculate, or arising as solitary branches from superficial hyphae, pale olivaceous to pale brown, paler towards the apex, irregular in width, straight to 1–2-geniculate, rarely branched, round to conical at the apex, 1–6-septate, 20.0–70.0(–230.0) × 2.5–3.5 μm. Conidial scars conspicuously thickened, 0.5–1.0 μm wide. Conidia cylindrical, catenate, later verruculose, subhyaline to pale olivaceous, straight to slightly curved, round to conical at the apex, obconical to obconically truncate at the base, 2–6-septate, 18.0–60.0 × 2.5–3.5 μm; hila small but conspicuously thickened.

Collection examined: **China. Taiwan Province**, Changhua County, on leaves of *Gynura bicolor* (Willd.) DC. (*Compositae*), 14 XII 1909, coll. K. Sawada (NYU-PPE, holotype).

Distribution: China.

Zasmidium bougainvilleae (J.M. Yen & Lim) Y.L. Guo, W.H. Hsieh & F.Y. Zhai, **comb. nov.** Fig. 2

MycoBank MB 808070

≡ *Stenella bougainvilleae* J.M. Yen & Lim, Mycotaxon 16: 96, 1982.

Leaf spots amphigenous, circular to subcircular, 1.0–4.0 mm diam., sometimes confluent, olivaceous-brown to pale brown, surrounded by a pale

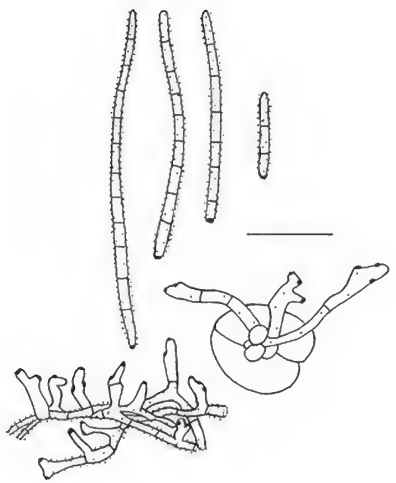


Fig. 2. *Zasmidium bougainvilleae* (HMAS 242906).
Conidiophores and conidia. Scale bar = 20 μm.

brown to grayish brown margin, with 1–2 times zonate and pale yellow to pale grayish brown halo on the upper surface, paler on the lower surface. Fruiting hypophyllous. Primary mycelium immersed. Secondary mycelium superficial; hyphae emerging through stomata or from the base of primary conidiophores, or directly from the apex of conidiophores, pale olivaceous to olivaceous, branched, septate, verruculose, with lateral secondary conidiophores, 1.5–3.0 μm diam. Stromata none or small, formed only by several brown cells. Primary conidiophores emerging through stomata, 2–8-fasciculate, or up to 25-fasciculate on small stromata, moderately olivaceous-brown to pale brown, paler towards the apex, irregular in width, narrower towards the apex, branched, straight to strongly curved, 0–3-geniculate, conical at the apex, 1–6-septate, sometimes constricted at the septa, 25.0–68.0 × 3.0–4.0 μm. Secondary conidiophores olivaceous-brown to moderately olivaceous-brown, unbranched, erect or curved, 0–3-septate, 15.0–48.0 × 2.5–4.0 μm. Conidial scars small but conspicuously thickened, 0.8–1.3 μm wide. Conidia cylindrical to slightly obclavate-cylindrical, pale olivaceous, catenate, straight to slightly curved, finely verruculose, indistinctly 2–14-septate, obtuse to conical at the apex, obconical to subtruncate at the base, with small but conspicuously thickened hila, 15.0–68.0(–110.0) × 2.0–3.5 μm.

Collections examined: **China. Hainan Province**, Lingshui, on leaves of *Bougainvillea glabra* Choisy (*Nyctaginaceae*), 11 IV 2011, coll. Y.L. Guo, no. HN345 (HMAS 242906). **Taiwan Province**, Changhua County, on leaves of *Bougainvillea spectabilis* Willd., 23 X 1985, coll. T.K. Goh (NCHUPP-184).

Distribution: China, Singapore.

Notes: Hsieh & Goh (1990: 248) described *Z. bougainvilleae* on *Bougainvillea spectabilis* from Taiwan as leaf spots absent or merely indefinite yellowish discoloration, with shorter conidiophores (primary conidiophores 20.0–40.0 × 3.0–5.0 μm, secondary conidiophores 5.0–15.0 × 2.0–4.0 μm), and longer conidia (20.0–110.0 × 2.5–4.0 μm). The morphological characters of our collection on *B. glabra* from Hainan are fairly similar to those described by Yen & Lim (1982) on *B. spectabilis* from Singapore (primary conidiophores 30.0–90.0 × 3.0–4.0 μm, conidia 20.0–65.0 × 2.5–4.0 μm).

Zasmidium salicis (Chupp & H.C. Greene) Kamal & U. Braun, Cercosporoid Fungi of India: 248, 2010.

≡ *Cercospora salicis* Chupp & H.C. Greene, Am. Midland Naturalist 41: 757, 1949.

≡ *Stenella salicis* (Chupp & H.C. Greene) Crous & U. Braun, Mycotaxon 78: 342, 2001.

Leaf spots amphigenous, punctiform to irregular, 0.5–3.0 mm diam., often angular, limited by veins, brown to blackish brown on the upper surface, blackish brown on the lower surface. Fruiting amphigenous. Mycelium internal. Stromata substomatal, subglobose, brown, 10.0–26.0 μm diam. Conidiophores arising from the upper cells of stromata, emerging through stomata, 3–25 per fascicle, pale olivaceous-brown, uniform in color, irregular in width, verruculose, straight to geniculate-sinuous, 0–4-geniculate, mostly unbranched, 0–2-septate, 8.0–28.0 × 2.5–5.0 μm. Conidial scars darkened and thickened, 2.0–2.5 μm wide. Conidia cylindrical, cylindrical-obclavate, solidary, pale olivaceous to olivaceous-brown, finely verruculose with age, straight to moderate curved, obtuse at the apex, subtruncate at the base, 1–7-septate, 15.5–96.0 × 2.5–5.0 μm, hila thickened and darkened.

Collection examined: **CHINA. Jilin Province**, Changchun, on leaves of *Salix matsudana* Koidz. (*Salicaceae*), 7 July 2006, coll. Y.J. Liu & F.Y.Zhai 6178 (HMJAU 30005).

Distribution: China, North America, Brazil.

Notes: This is the first report of *Zasmidium salicis* from China.

Chupp & Greene described *Cercospora salicis* from North America on *Salix alba* L. (Greene 1949; Chupp 1954). The holotype was re-examined by Crous & Braun (2001), who reallocated it to *Stenella* based on verruculose superficial hyphae and conidia. Kamal and Braun subsequently transferred the species to *Zasmidium* (Kamal 2010).

The conidiophores and conidia of our Chinese specimen are fairly similar to those of *Z. salicis* on *Salix* sp. in North America in shape and size but differ in lacking verruculose external mycelium, with the North American material having larger stromata (≤50 μm wide), 1–3-septate and somewhat

larger conidiophores (15–45 × 4–6 μm), and 1–6-septate and slightly smaller conidia (20–60 × 3–4.5 μm). The development of verruculose superficial hyphae is a basic feature of *Zasmidium* and well developed in most species, but may occasionally be absent (Crous & Braun 2003). A few species without any superficial mycelium have been assigned to this genus, e.g., *Zasmidium hymenocallidis* (U. Braun & Crous) U. Braun & Crous and *Z. macluricola* R.G. Shivas et al. (Shivas et al. 2009, Braun et al. 2010a).

Mycovellosiella salicis Deighton et al. (Deighton 1974) parasitic on *Salix tetrasperma* Roxb. is morphologically similar to *Zasmidium salicis* but differs in lacking obvious leaf spots and stromata and forming hypophyllous and diffusely fruiting, paler (pale olivaceous), and longer (≤80 μm) conidiophores, and paler (pale olivaceous) catenate conidia. *Mycovellosiella salicis* has been reallocated to *Passalora salicis* (Deighton et al.) U. Braun & Crous (Crous & Braun 2003).

Acknowledgments

The study was financed by the "Knowledge Innovation Program" of the Chinese Academy of Sciences – Flora Cryptogamarum Sinicarum Compilation and Research Foundation (Grant No. KSCXZ-EW-Z-9) and the National Natural Science Foundation of China (Grant No. 31100013). We express our deep appreciation to Dr. Uwe Braun and Shu-Yan Liu for their valuable suggestions, kind help, and their earnest assistance in the course of submission of this manuscript.

Literature cited

Braun U, Crous PW, Schubert, K, Shin, H-D. 2010a. Some reallocations of *Stenella* species to *Zasmidium*. Schlechtendalia 20: 99–104.

Braun U, Freire F, Urtiaga R. 2010b. New species and new records of cercosporoid hyphomycetes from Brazil, New Zealand and Venezuela. Polish Botanical Journal 55: 281–291.

Crous PW, Braun U. 2001. A reassessment of the *Cercospora* species described by C. Chupp: specimens deposited at BPI, Maryland, U.S.A. Mycotaxon 78: 327–343.

Crous PW, Braun U. 2003. *Mycosphaerella* and its anamorphs: 1. Names published in *Cercospora* and *Passalora*. CBS Biodiversity Series No. 1. 569 p.

Deighton FC. 1974. Studies on *Cercospora* and allied genera. V. *Mycovellosiella* Rangel, and a new species of *Ramulariopsis*. Mycological Papers 137. 76 p.

Greene HC. 1949. Notes on Wisconsin parasitic fungi XIII. The American Midland Naturalist 41: 740–758. http://dx.doi.org/10.2307/2421783

Hsieh WH, Goh TK. 1990. *Cercospora* and similar fungi from Taiwan. Maw Chang Book Company. Taichung Taiwan R.O.C. 367 p.

Kamal. 2010. Cercosporoid fungi of India. Dehra Dun: Bishen Singh Mahendra Pal Singh. 351 p.

Shivas RG, Young AJ, Braun U. 2009. *Zasmidium macluricola*. Fungal Planet 39. Persoonia 23: 190–191.

Yen JM, Lim G. 1982. Studies on parasitic fungi from South East Asia, 45. Parasitic fungi from Malaysia, 22. Mycotaxon 16(1): 96–98.

ISSN (print) 0093-4666 © 2014. Mycotaxon, Ltd. ISSN (online) 2154-8889

MYCOTAXON

http://dx.doi.org/10.5248/129.63

Volume 129(1), pp. 63–72 *July–September 2014*

New reports of *Gymnopus* from Pakistan based on ITS sequences

M. Saba* & A.N. Khalid

Department of Botany, University of the Punjab
Quaid-e-Azam Campus, Lahore, 54590, Pakistan

* Correspondence to: *rustflora@gmail.com*

Abstract — Specimens of *Gymnopus luxurians* and *G. menehune* from Pakistan are characterized morphologically and using nrDNA internal transcribed spacer sequences. These fungal species represent new records for South Asia. A key to the *Gymnopus* species known from Pakistan is provided.

Key words — Abbottabad, *Collybia*, Khyber Pakhtoonkhaw, *Marasmiaceae*, saprobic fungi

Introduction

Gymnopus (Pers.) Roussel was monographed by Antonín & Noordeloos (1997) and many mushrooms from *Collybia* sections *Vestipedes* and *Levipedes* have now been transferred into this genus (Antonín & Noordeloos 1997). *Gymnopus* consists of delicate to semi-fleshy mushrooms commonly found on leaf and woody litter in tropical to temperate ecosystems, and *Gymnopus* sect. *Vestipedes* is characterised by a hairy or tomentose stipe surface and a simple pileus epicutis without a Rameales- or Dryophila-structure (Antonín & Noordeloos 1997). Ecologically, *Gymnopus* species are considered important in recycling nutrients (Singer 1986; Mata & Ovrebo 2009).

Five *Gymnopus* species have previously been reported from Pakistan (under their *Collybia* synonyms): *Gymnopus confluens* (Pers.) Antonín et al., *G. dryophilus* (Bull.) Murrill, *G. fusipes* (Bull.) Gray, *G. erythropus* (Pers.) Antonín et al. (as *C. kuehneriana*), and *G. peronatus* (Bolt.) Gray (Ahmad 1980; Iqbal & Khalid 1996; Shibata 1992; Sultana et al. 2011). During a collecting trip for macrofungi in Khyber Pakhtoonkhaw in 2012, many mushroom species were collected. Amongst them, three specimens belonging to *Gymnopus* sect. *Vestipedes* were identified as *G. luxurians* and *G. menehune*, which are new records from subtropical pine forests of Pakistan.

Table 1. ITS-rDNA sequences of *Gymnopus* and *Omphalotus* spp. used in the phylogenetic analysis

Taxon	Country	Genbank #	Collection/ Voucher #
G. biformis	Costa Rica	DQ450064	TFB7843
	Costa Rica	DQ450063	TFB7820
G. confluens	Russia	AY256697	TENN58242
	—	HM240527	UBC F19677
G. cylindricus	Costa Rica	AY256696	TENN58024
	Costa Rica	DQ450057	TENN58024
G. dryophilus	Italy	JX536157	BRNM707149
	Czech Republic	JX536158	BRNM712600
G. erythropus	Slovakia	DQ449996	SAV XI2002
	USA	DQ449998	WTU JFA12910
G. fibrosipes	Costa Rica	AF505763	TENN56660
	—	AY842953	PR23TN
G. fusipes	France	AY256710	TENN59217
	Austria	AF505777	TENN59300
G. gibbosus	—	AY263437	AWW66
	—	AY263438	AWW95
G. luxurians	Pakistan	KF803760	MSM#001
	Pakistan	KF803761	MSM#002
	USA	AY256709	TENN57910
	Switzerland	DQ450022	TENN50619
G. menehune	Pakistan	KF803762	MSM#003
	—	JN182864	Not given
	—	AY263426	DED5866
G. mesoamericanus	Costa Rica	DQ450036	TENN58106
	Costa Rica	AF505768	NYBG REH7379
G. polygrammus	Puerto Rico	DQ450028	TENN56589
	Not given	AY842954	PR 2542TN
G. readiae	New Zealand	DQ450034	TENN53687
	New Zealand	HQ533036	PDD95844
G. subcyathiformis	Puerto Rico	DQ450041	TENN58130
	Dominican Republic	DQ450042	TENN59550
G. subpruinosus	USA	DQ450026	TENN56242
	USA	DQ450027	TENN59477
O. illudens	USA	AY313271	TENN54507
O. olearius	France	AY313277	culture 9061b
O. olivascens	USA	AY313281	TENN55337

Materials & methods

Basidiomata were collected, photographed, vouchered, dried under fan heater, and characterized morphologically. Specimen sections were mounted in 5% KOH for observation under a biological microscope (MX4300H, Meiji Techno Co., Ltd., Japan). Phloxine was used to increase contrast of structures, and Melzer's reagent was used to test for dextrinoidity of basidiospores.

Measurements of anatomical features (basidiospores, basidia, cystidia, pileus hyphae, and stipe hyphae) are presented from at least 20 measurements made with an ocular micrometer and 100× oil-immersion objective; basidiospore abbreviations include x = arithmetic mean of length and width for all spores measured and Q = length divided by width. Line drawings were made with a camera lucida. Color designations are from Munsell (1975) colour system.

Genomic DNA was extracted from a small piece of pileus by a modified CTAB method (Bruns 1995). The internal transcribed spacers (ITS1 and ITS2) and 5.8S region of the nuclear ribosomal RNA gene were amplified with the primer pairs ITS1F/ITS4 (White et al. 1990; Gardes & Bruns 1993) using the Extract-N-Amp plant DNA extraction Kit (Sigma-Aldrich, St. Louis, MO, USA). PCR was carried out under the following cycling parameters: initial denaturation (94 °C for 1 min), 35 cycles (94 °C for 1 min, 53 °C for 1 min, and 72 °C for 1 min), and final extension 72 °C (8 min). Amplified PCR products were sent for purification and bidirectional sequencing to Macrogen (Korea). Two sequences each of *Gymnopus luxurians* and *G. menehune* and other related sequences were retrieved from the Genbank and aligned by Muscle using the default setting in Molecular Evolutionary Genetics Analysis (MEGA) software (Tamura et al. 2011). Sequences were manually edited and assembled using BioEdit (www.mbio.ncsu.edu/bioedit/bioedit.html). Following Dentinger et al. (2011) for complete ITS sequences, all sequences were trimmed with the conserved motifs 5′-(… GAT) CATTA– and –GACCT (CAAA…)-3′ and the alignment portion between them was included in the analysis. All positions containing gaps and missing data were eliminated. A phylogenetic tree was constructed with the Maximum Likelihood (ML) algorithm and Jukes & Cantor (1969) model of nrITS sequences and nearest-neighbor-interchange (NNI) as ML heuristic search method using MEGA5 software (Tamura et al. 2011). Phylogeny was tested by bootstrap value of 1000 replicates, and corresponding bootstrap values >50 % are cited in the tree. Sequences of *Omphalotus* spp. were used as outgroup based on results reported by Moncalvo et al. (2002) and Mata et al. (2004).

Sequences generated for this study were submitted to Genbank. A complete list of taxa used in this phylogenetic is shown in Table 1. Percent identities (PID) and DNA divergence were calculated by DNAStar.

Taxonomy

Gymnopus luxurians (Peck) Murrill, N. Amer. Fl. 9(5): 362 (1916). Fig. 1

Pileus 19–29 mm diam, convex when young, expanding with age to plane, flat, thin (collybioid), slightly umbonate; margin recurved or uplifted, flexuous, striate; surface dull, fibrillose; brown, dark brown or reddish brown (10R5/10), margin fading to pale brown to brown or buff (10R6/12). Lamellae adnexed,

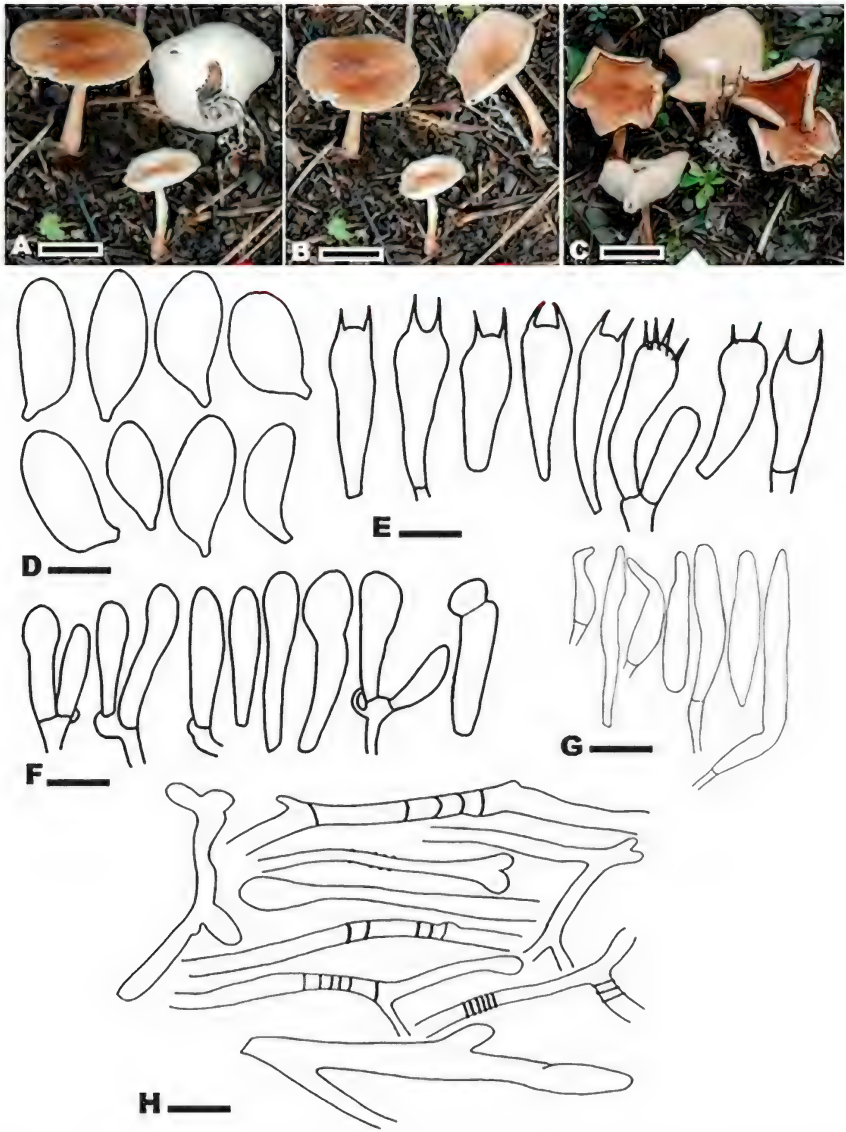


Fig. 1. *Gymnopus luxurians* (MSM#001; MSM #002). (A–C) Basidiomes. (D) Basidiospores. (E) Basidia and basidioles. (F) Cheilocystidia. (G) Caulocystidia. (H) Pileipellis. Scale bars: A–C = 2 cm; D–F = 10 µm; G–H = 20 µm.

indented or notched, close to subdistant, white at first (5YR9/2), pale grayish (5YR8/6) at maturity. Stipe 13–25 mm, central, subequal, often with subbulbous

base, hollow, striate, buff (2.5YR6/8) when young, base dark brown (10R2/6). Rhizomorphs white, numerous, branched. Odor and taste not recorded. Basidiospores 4.2–5 × 5.3–9.4 µm [x = 4.6 × 7.6 µm, Q = 1.65], oblong to ellipsoid in profile, apiculus present, smooth, thin-walled, hyaline in KOH, inamyloid. Basidia 5.6–8 × 19–28.3 µm, clavate, 2-spored basidia abundant, 4-spored also present, thin-walled, hyaline in KOH; sterigmata 2–4 µm. Basidioles subclavate, abundant. Pleurocystidia absent. Cheilocystidia 4–8 × 18–32 µm, clavate or cylindrical, hyaline, thin-walled. Pileipellis a cutis, hyphae cylindrical, 6–14 µm, thin-walled, weakly to heavily encrusted with annular brown pigments, brown in KOH. Stipe hyphae cylindrical, 5–10 µm, non-encrusted, hyaline to pale yellow in KOH. Caulocystidia cylindrical, hyaline, thin-walled 9–14 × 53–124 µm. Clamp connections present in all tissues.

Material examined: **PAKISTAN, Khyber Pakhtoonkhaw**, Abbottabad, Shimla, under *Pinus roxburghii* Sarg., 14 September 2012, Malka Saba & Abdul Nasir Khalid, MSM #001 (LAH; GenBank KF803760); MSM #002 (LAH; GenBank KF803761).

Comments: *Gymnopus luxurians* is a widely distributed species. It is characterized by brown or reddish brown convex pileus, slightly umbonate with striate margin, oblong to ellipsoid basidiospores, and cylindrical caulocystidia. It is frequently collected on woody debris across the continental United States and has been reported from Europe (Breitenbach & Kränzlin 1991), Hawaii (Desjardin et al. 1999), Dominican Republic (Mata et al. 2006), Costa Rica, and Panama (Mata & Ovrebo 2009). *Gymnopus luxurians* represents a new record for Pakistan.

Gymnopus luxurians falls in *G.* subsect. *Vestipedes* (Antonín & Noordeloos 1997). Other *Gymnopus* species from this subsection reported from Pakistan include *G. confluens*. (Ahmad 1980; Shibata 1992; Sultana et al. 2011), *G. peronatus* (Sultana et al. 2011), and *G. menehune* (see below). *Gymnopus confluens* is similar to *G. luxurians* in being reported from pine forests during the same season of the year but can be separated by the presence of thin and crowded lamellae and a whitish fuzz on the stipe that becomes noticeable as the fungus matures (Kuo 2013). *Gymnopus peronatus* can be distinguished by its remarkably distant gills and densely woolly-strigose stipe with white to yellow hairs (Antonín & Noordeloos 1997).

Gymnopus menehune Desjardin, Halling & Hemmes, Mycologia 91(1): 173 (1999). Fig. 2

Pileus 10–14 mm diam., convex to plano-convex and slightly umbilicate when young, infundibuliform, umbilicate at maturity, reddish brown (10R3/8) or brown fading near margin to grey or pale brown (5YR9/2); margin decurved, straight or flaring, uplifted, eroded, striate; surface dull, glabrous. Lamellae adnate to decurrent, ascending, close, with 3–6 series of lamellulae,

off-white to pale orange white (10R8/4). Stipe 23–32 mm, central, equal, hollow, pubescent to tomentose, buff color near proximal end, brown to dark brown (10R2/6) at distal end. Rhizomorphs numerous, white, branched. Odor and taste not recorded. Basidiospores elongate to ellipsoid in profile, 3–4.2(–5.4) × (–5.5)7–9 µm [x = 4.6 × 8.1 µm, Q = 1.76], smooth, thin-walled, hyaline in KOH with cytoplasmic contents, inamyloid. Basidia clavate, 6.3–7(–8) × 18.8–24 µm, commonly 2-spored, occasionally 4-spored; Sterigmata up to 4.9 µm. Basidioles clavate or fusoid, abundant. Pleurocystidia absent. Cheilocystidia broadly clavate or irregular in outline, sometimes lobed, thin-walled, 5–7 × 20–30 µm, rarely observed. Pileipellis a cutis, hyphae 3.7–6.3 diam., cylindrical with outgrowths or a few diverticula, thin-walled, weakly encrusted with brown pigments, terminal cells non-encrusted, lobed, pale brown in KOH, inamyloid. Stipe hyphae 5.9–6.4 µm, cylindrical, parallel, thin-walled, hyaline to pale brown in KOH, inamyloid. Caulocystidia 5–6 × 31–54 µm, cylindrical to sinuous, hyaline, thin-walled. Clamp connections present in all tissues.

Material examined: **PAKISTAN**, **Khyber Pakhtoonkhaw**, Mansehra, Dadar, under *Pinus roxburghii*, 15 September, 2012, Malka Saba & Abdul Nasir Khalid, MSM #003 (LAH; GenBank KF803762).

Comments: *Gymnopus menehune* is diagnosed by its convex to plano-convex, umbilicate pileus, pubescent to tomentose stipe, and elongate to ellipsoid basidiospores. According to Desjardin et al. (1999) there are voluminous lobed cheilocystidia in *G. menehune*, but cheilocystidia were rarely observed in our Pakistan collection. The species was described first from Hawaii (Desjardin et al. 1999) and later from Indonesia (Wilson et al. 2004). *Gymnopus menehune* represents a new record for Pakistan.

Gymnopus menehune falls in *G.* subsect. *Vestipedes* (Antonín & Noordeloos 1997).

Phylogenetic analysis

Sequencing of the nrITS region of *Gymnopus luxurians* yielded fragments of 800 base pairs while *G. menehune* yielded 830 base pairs. Initial BLAST analysis of nucleotide sequences showed collections MSM#001 and MSM#002 with 100% and 99% maximum identity with *Gymnopus luxurians* (GenBank AY256709, DQ480106, DQ450022), while collection MSM#003 showed 97% maximum identity with *G. menehune* (GenBank JN182864, AY263426, AY263444, DQ450043, AY263443).

Sequences of closely related taxa were retrieved from Genbank for phylogenetic analysis. In addition to the three new Pakistani sequences of *G. luxurians* and *G. menehune*, the analysis included 30 other *Gymnopus* sequences from GenBank, and three *Omphalotus* sequences as outgroup (Table 1).

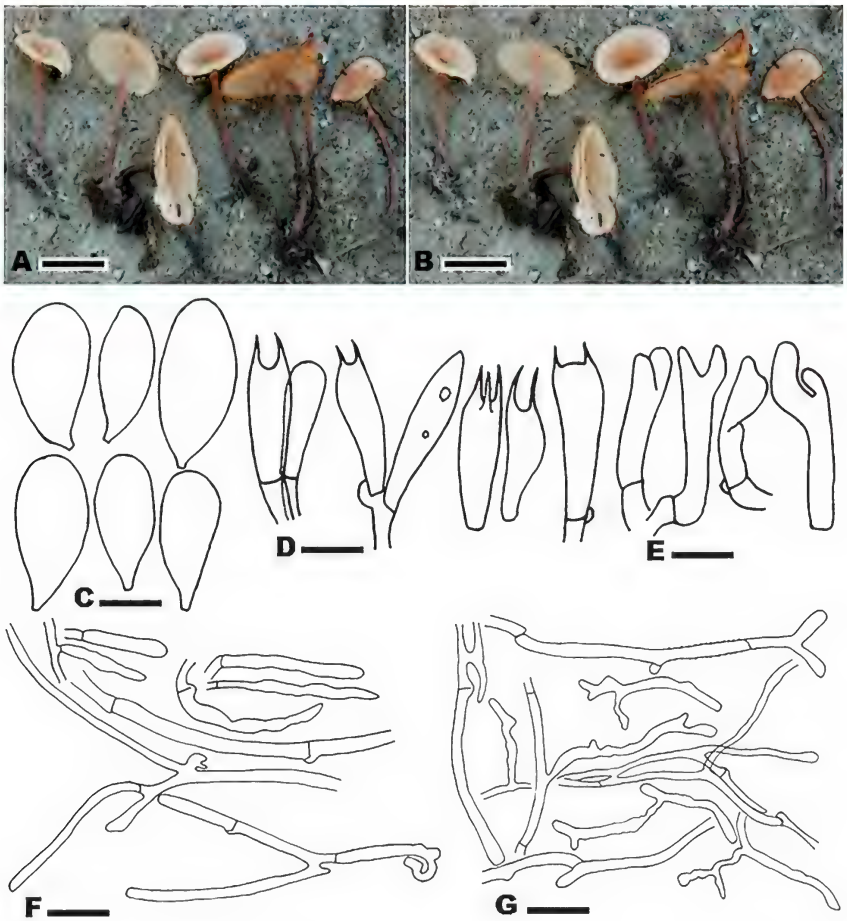


Fig. 2. *Gymnopus menehune* (MSM #003). (A–B) Basidiomes. (C) Basidiospores. (D) Basidia and basidioles. (E) Cheilocystidia. (F) Stipe elements and caulocystidia. (G) Pileipellis. Scale bars: A–B = 2 cm; C–E = 10 μm; F–G = 20 μm.

After removing and editing ambiguities from the aligned sequences, a total of 948 positions were in the final dataset. Of these, 508 characters were conserved, 418 were variable, 380 were parsimony informative, and 32 were singletons.

Clade I, and II (Fig. 3) included species of *Gymnopus* subsect. *Vestipedes*, Clade III species characteristic of *G.* sect. *Gymnopus*, and clade IV species of *G.* subsect. *Levipedes*. Pakistani sequences (▪) generated in this study clustered with their respective morphological species, each one with strong bootstrap value. *Gymnopus luxurians* appears in a clade distinct from *G. menehune*.

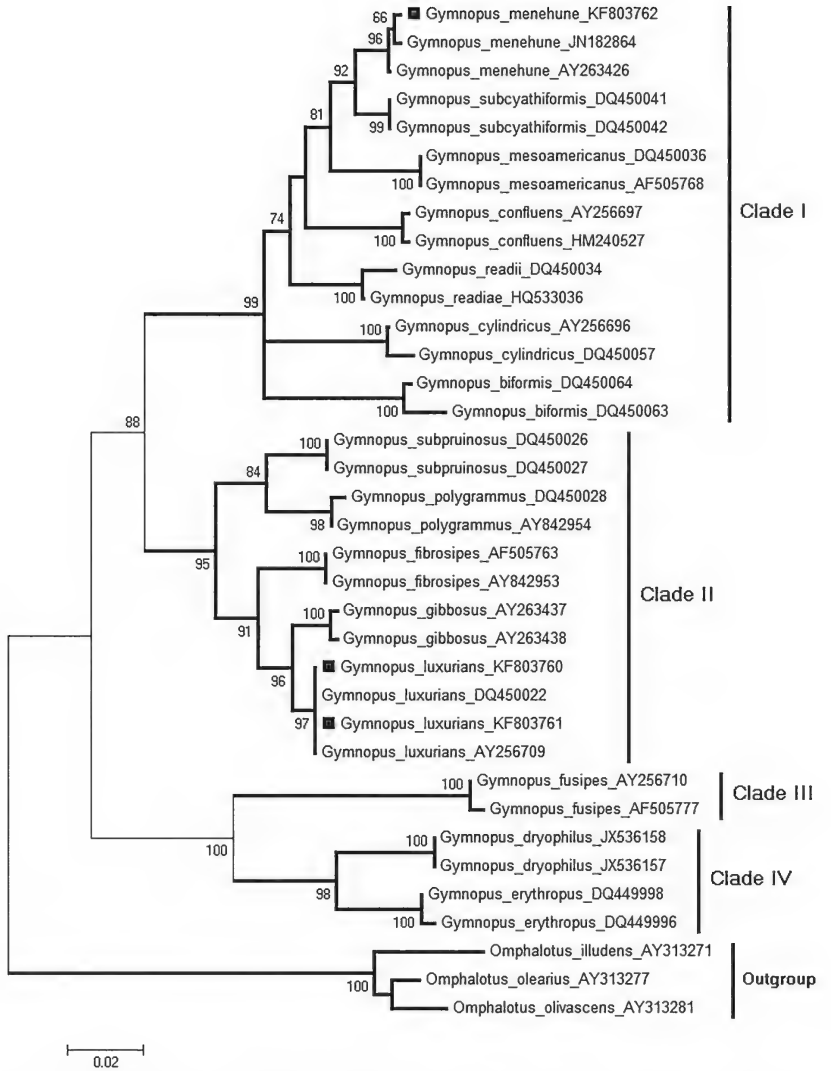


FIG. 3. Molecular phylogenetic analysis of *Gymnopus* sequences by the Maximum Likelihood method. The bootstrap consensus tree inferred from 1000 replicates is taken to represent the evolutionary history. The percentage of trees in which the associated taxa clustered together is shown next to the branches. New sequences reported in this study (▪).

The percentage similarity, calculated by DNAStar, of *Gymnopus luxurians* showed 100% identity and 0% genetic divergence with *G. luxurians* sequences AY256709 and DQ450022, and *G. menehune* showed 99.9% identity and

0.1% divergence with *G. menehune* sequences JN182864 and AY263426; this supports the results of the BLAST analysis and the phylogenetic tree.

Key to the *Gymnopus* spp. in Pakistan

1. Pileipellis a transition between cutis and trichoderm and comprising lobed (often coralloid) elements, similar to a Dryophila-structure ..... *G. fusipes*
1. Pileipellis usually a simple cutis with some weakly to distinctly coralloid or diverticulate terminal elements, without Dryophila-structure 2
2. Stipe smooth 3
2. Stipe hairy or tomentose 4
3. Pileus dark red brown at centre, much paler yellow brown to yellow red towards margin ..... *G. erythropus*
3. Pileus with ochre brown tinges, especially at centre, translucently striate up to half the radius ..... *G. dryophilus*
4a. Pileus convex when young, expanding with age to plane, flat, thin (collybioid), slightly umbonate; lamellae adnexed, close to subdistant; basidiospores oblong to ellipsoid, 4–5 × 5.5–9.5 µm ..... *G. luxurians*
4b. Pileus convex to plano-convex and slightly umbilicate when young, infundibuliform, umbilicate at maturity; lamellae adnate to decurrent, close; basidiospore elongate to ellipsoid, 3–5.5 × 5.5–9 µm ..... *G. menehune*
4c. Pileus convex with an incurved margin when young, becoming broadly convex, bell shaped or nearly flat; lamellae narrowly attached to the stem or free, crowded or close; basidiospores lacrymoid to elliptical or nearly fusoid, 3.5–5 × 6–10 µm ..... *G. confluens*
4d. Pileus convex then flattened, often with a low, broad umbo, tan to darker brown; lamellae adnexed to free, crowded; basidiospores elongate, 3–4 × 7–9 µm ..... *G. peronatus*

Acknowledgments

We are cordially grateful for Higher Education Commission (HEC) for funding this project under Phase II, Batch I, Indigenous PhD fellowships program for 5000 scholars. We are highly indebted to Dr. Juan Luis Mata (Department of Biology, University of South Alabama, USA) and Dr. Amy Rossman (Systematic Mycology and Microbiology Laboratory, USDA-ARS, Beltsville, USA) for critically reviewing the manuscript. We are thankful to all lab fellows for accompanying us on the field trip. I am cordially thankful to Dr. Samina Sarwar (Lahore College for Women University) for her help and guidance preparing the phylogeny.

Literature cited

Ahmad S. 1980. A contribution to the *Agaricales* of Pakistan. Bulletin of Mycology 1: 35–89.

Antonín V, Noordeloos M. 1997. A monograph of *Marasmius, Collybia* and related genera in Europe. Part 2: *Collybia, Gymnopus, Rhodocollybia, Crinipellis, Chaetocalathus* and additions to *Marasmiellus*. Libri Botanici 17. 256 p.

Breitenbach J, Kränzlin F. 1991. Fungi of Switzerland. Vol. 3. Boletes and agarics, first part. Verlag Mykologia, Luzern. 361 p.

Bruns TD. 1995. Thoughts on the processes that maintain local species diversity of ectomycorrhizal fungi. Plant and Soil 170: 63–73. http://dx.doi.org/10.1007/BF02183055

Dentinger BTM, Didukh MY, Moncalvo JM. 2011. Comparing COI and ITS barcode markers for mushrooms and allies (*Agaricomycotina*). PLoS One 6(9): e25081. http://dx.doi.org/10.1371/journal.pone.0025081

Desjardin DE, Halling RE, Hemmes DE. 1999. *Agaricales* of the Hawaiian Islands. 5. The genera *Rhodocollybia* and *Gymnopus*. Mycologia 91: 166–176. http://dx.doi.org/10.2307/3761206

Gardes M, Bruns TD. 1993. ITS primers with enhanced specificity for *Basidiomycetes*: application to the identification of mycorrhizae and rusts. Molecular Ecology 2: 113–118. http://dx.doi.org/10.1111/j.1365-294X.1993.tb00005.x

Iqbal SH, Khalid AN. 1996. Materials for the fungus flora of Pakistan. I. Checklist of agarics, their distribution and association with the surrounding vegetation. Science International (Lahore) 8(1): 51–64.

Jukes TH, Cantor CR. 1969. Evolution of protein molecules. 21-132, in: HN Munro (ed.). Mammalian Protein Metabolism. Academic Press, New York.

Kuo M. 2013. *Gymnopus confluens*. Retrieved from the MushroomExpert.com. http://www.mushroomexpert.com/gymnopus_confluens.html

Mata JL, Ovrebo CL. 2009. New reports and illustrations of *Gymnopus* for Costa Rica and Panama. Fungal Diversity 38: 125–131.

Mata JL, Hughes KW, Petersen RH. 2006. An investigation of *Omphalotaceae* (*Fungi:* Euagarics) with emphasis on the genus *Gymnopus*. Sydowia 58: 191–289.

Moncalvo JM, Vilgalys R, Redhead SA, Johnson JE, James TY, Aime MC, Hofstetter V, Verduin SJW, Larsson E, Baroni TJ, Thorn RG, Jacobsson S, Clémençon H, Miller OK. 2002. One hundred and seventeen clades of Euagarics. Molecular Phylogenetics and Evolution 23: 357–400.

Munsell ™. 1975. Soil color charts. Baltimore.

Shibata H. 1992. Higher basidiomycetes from Pakistan. Cryptogamic Flora of Pakistan 1: 145–164.

Singer R. 1986. The *Agaricales* in modern taxonomy. 4th edn. Koeltz, Koenigstein. 980 p.

Sultana K, Rauf CA, Riaz A, Naz F, Irshad G, Haque MI. 2011. Checklist of agarics of Kaghan valley – 1. Pakistan Journal of Botany 43(3): 1777–1787.

Tamura K, Peterson D, Peterson N, Stecher G, Nei M, Kumar S. 2011. Molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. Molecular Biology and Evolution 28(10): 2731–2739. http://dx.doi.org/10.1093/molbev/msr121

White TJ, Bruns T, Lee S, Taylor JW. 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. 315–322, in: MA Innis et al. (eds). PCR Protocols: a guide to methods and applications. Academic, New York.

Wilson AW, Desjardin DE, Horak E. 2004. *Agaricales* of Indonesia. 5. The genus *Gymnopus* from Java and Bali. Sydowia 56(1): 137–210.

ISSN (print) 0093-4666 © 2014. Mycotaxon, Ltd. ISSN (online) 2154-8889

MYCOTAXON

http://dx.doi.org/10.5248/129.73

Volume 129(1), pp. 73–77 *July–September 2014*

First report of *Callistosporium luteoolivaceum* from Western Himalaya, Pakistan

M. Saba* & A.N. Khalid

Department of Botany, University of the Punjab
Quaid-e-Azam Campus, Lahore, 54590, Pakistan

* Correspondence to: *rustflora@gmail.com*

Abstract — *Callistosporium luteoolivaceum* was collected from pure pine forests of Western Himalaya, Pakistan, and identified based on morphological characters and nrDNA ITS sequences. It is characterized by dull yellow or olive yellow pilei, lamellae, and stipes that turn reddish brown with age and basidiospores that turn purplish in KOH. This species is new to the mycobiota of Pakistan.

Key words — Abbottabad, *Basidiomycota,* Khyber Pakhtoonkhaw, saprobic fungi, *Tricholomataceae*

Introduction

Geographically, the areas of Pakistan and Indian-held Kashmir fall within the Western Himalaya. The climate ranges from tropical at the base of the mountains to permanent ice and snow at the highest elevations. The Himalayan forests have been reliably identified as a 'biodiversity hotspot', a global priority for the conservation of biodiversity (Wikramanayake et al. 2002; Qamar et al. 2011).

During a 2012 mushroom collecting trip in pure pine forests in the Himalayan range, many species of saprobic macrofungi were collected. Amongst them, *Callistosporium luteoolivaceum* was identified morphologically and molecularly as a new record from Pakistan. This very variable species occurs worldwide in temperate or tropical forests mostly outside of boreal and subalpine regions (Redhead 1982). According to Kuo (2006) this little mushroom is a decomposer found primarily on dead conifer wood. No *Callistosporium* species has previously been reported from Pakistan.

Materials & methods

Basidiomata were collected, photographed, vouchered, dried under fan heater and characterized morphologically. Specimen sections were mounted in 5% KOH for

observation under a MX4300H biological microscope (Meiji Techno Co., Ltd., Japan). Phloxine was used to increase contrast of structures, and Melzer's reagent was used to test for dextrinoidity of basidiospores.

Measurements of morphological features (basidiospores, basidia, cystidia, and pileus and stipe hyphae) were taken from at least 20 measurements made with an ocular micrometer and 100× oil-immersion objective, where x = arithmetic mean of spore length and width for all spores measured, and Q = spore length divided by spore width. Line drawings were made with camera lucida. Color designations are from Munsell (1975). The collection was conserved in the Herbarium of the Department of Botany, University of the Punjab, Lahore, Pakistan (LAH).

Genomic DNA was extracted from a small piece of pileus by a modified CTAB method (Bruns 1995). The internal transcribed spacers (ITS1 and ITS2) and the 5.8S nuclear ribosomal gene were amplified with primer pairs ITS1F/ITS4 (White et al. 1990; Gardes and Bruns 1993) using the Extract-N-Amp plant DNA extraction Kit (Sigma-Aldrich, St. Louis, MO, USA). PCR cycling parameters were as follows: initial denaturation (94 °C for 1 min), 35 cycles (94 °C for 1 min, 53 °C for 1 min, and 72 °C for 1 min), and final extension 72 °C (8 min). Amplified PCR products were sent for purification and bidirectional sequencing to Macrogen (Republic of Korea). One *C. luteoolivaceum* sequence and other related sequences were retrieved from GenBank and aligned by Muscle using default setting in Molecular Evolutionary Genetics Analysis (MEGA) software (Tamura et al. 2011). Sequences were manually edited and assembled using BioEdit (www.mbio.ncsu.edu/bioedit/bioedit.html). Following Dentinger et al. (2011) for complete ITS sequences, all sequences were trimmed with the conserved motifs 5′-(...GAT) CATTA– and –GACCT (CAAA...)-3′ and the alignment portion between them were included in analysis. A sequence generated for this study was submitted to GenBank. Percent Identities (PID) and DNA divergence were calculated by Megalign (DNA Star Inc.).

Taxonomy

Callistosporium luteoolivaceum (Berk. & M.A. Curtis) Singer, Lloydia 9: 117 (1946). Figs 1, 2

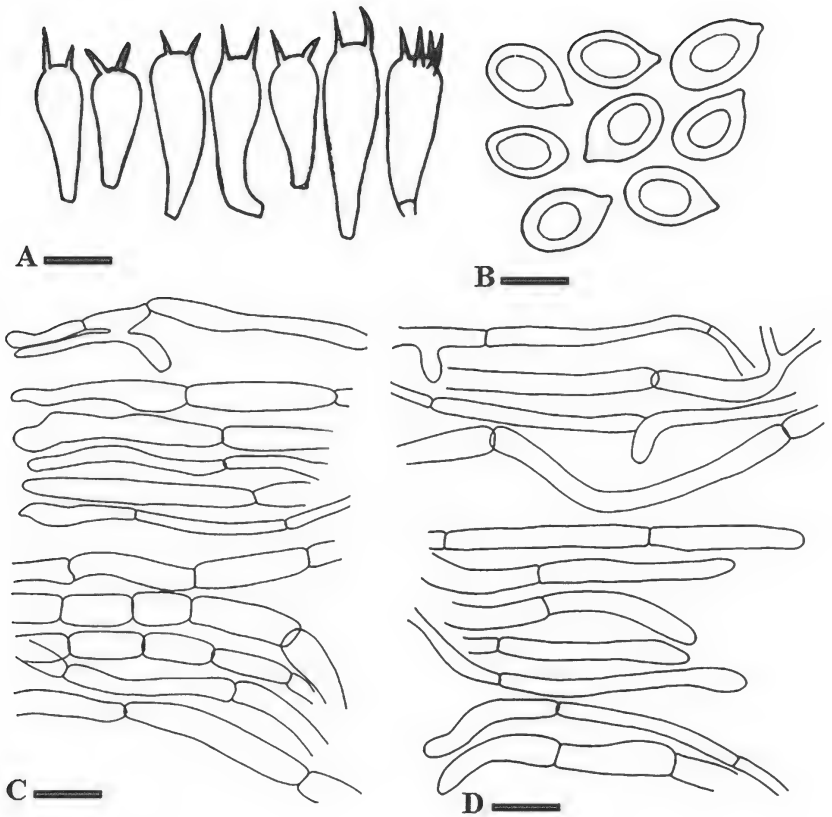
Pileus 27–31 mm diam., convex to plano-convex, flat, thin (collybioid); margin straight or flaring, smooth; surface dull, smooth, olive yellow near margin (5YR7/10); disc moderately indented, dark brown (5YR1/4) at the center. Lamellae regular, adnate, close to crowded, pale yellow or olive yellow (5YR7/8); margin wavy. Stipe 55–58 mm, central, equal, hollow, striate, pale yellow to olive yellow (5YR7/8); annulus absent; volva absent. Rhizomorphs white, few. Odor and taste not recorded. Basidiospores 4.6–6.6 × 3–4.5 µm [x = 5.2 × 3.8 µm, Q = 1.36], ellipsoid in profile, smooth, thin-walled, purplish in KOH, apiculus present; Basidia 16.9–24.5 × 5.4–7.8 µm, clavate, two to four spored, thin-walled, some turning deep purple in KOH; sterigmata 2.8–4.2 µm. Trama hyphae, parallel, thin-walled, hyaline in KOH. Cheilocystidia 12–26.6 × 4.7–8 µm, clavate or cylindrical, hyaline, thin-walled. Pleuro-

Callistosporium luteoolivaceum (MSM #004, LAH). Basidiomes. Scale bars = 7 mm.

CYSTIDIA absent. PILEIPELLIS a cutis; hyphae cylindrical, 4–6 µm, thin-walled, hyaline in KOH, some with brown pigments; pileal cystidia clavate, 34–61 × 5.1–8.2 µm, thin walled, hyaline in KOH. STIPE hyphae cylindrical, 3.5–17 µm, hyaline in KOH. All structures inamyloid. CLAMP CONNECTIONS absent.

MATERIAL EXAMINED: **PAKISTAN**, **KHYBER PAKHTOONKHAW**, Abbottabad, Shimla, under *Pinus roxburghii* Sarg., 14 September 2012, MSM #004 (LAH; GenBank KJ101607).

DISCUSSION: *Callistosporium luteoolivaceum* was first described from eastern North America. It is taxonomically complicated, and its variable characters and wide world distribution has resulted in its being described many times as a new taxon. Redhead (1982) critically examined *C. elaeodes* Bon, *C. favrei* Singer, *C. graminicolor* Lennox, *C. luteofuscum* Singer, *C. luteofuscum* var. *major* Singer, *C. majus* Singer, and *C. xanthophyllum* (Malençon & Bertault) Bon and reduced them to synonymy under *C. luteoolivaceum* because their distinguishing features intergrade. Despite the intergradations of critical characters, there remains a great diversity of forms within the broad species concept which when viewed individually appear quite distinct from one another (Redhead 1982).



Callistosporium luteoolivaceum (MSM #004, LAH).
(A) Basidia. (B) Basidiospores. (C) Stipe elements. (D) Pileipellis and pileal cystidia.
Scale bars: A = 10 µm; B = 4.5 µm; C, D = 17.5 µm.

Our collection is in remarkable agreement with the description provided by Redhead (1982). This is the first report of *C. luteoolivaceum* from Pakistan. It has been reported from North and South America, Europe, and Asia (Hongo 1981; Horak 1987; Manimohan & Leelavathy 1989; Redhead 1982; Singer 1970). This species is listed among the rare and notable macrofungi of British Columbia (Redhead 1997: 6–8).

Sequencing of the nrITS region of *C. luteoolivaceum* yielded fragments of 690 base pairs when its PCR product was sequenced. Initial blast analysis showed our Pakistani sequence (GenBank KJ101607) with 97% maximum identity with *C. xanthophyllum* (GenBank JF907781; AF325667) and *C. luteoolivaceum* (GenBank AF325666). Further analysis reinforced the blast results, showing

97.5% identity and 0.5% genetic divergence between our Pakistani sequence and the *C. luteoolivaceum* sequence (GenBank AF325666).

Acknowledgments

We are highly indebted to Higher Education Commission (HEC), Pakistan, for funding this project under Phase II, Batch I, Indigenous PhD fellowships program for 5000 scholars. We are cordially grateful to Dr. Juan Luis Mata (Dept. of Biology, University of South Alabama, USA) and Dr. Vladimír Antonín (Moravian Museum, Czech Republic) for critically reviewing the manuscript and their valuable comments. We are thankful to all lab fellows for accompanying us on field trip.

Literature cited

Bruns TD. 1995. Thoughts on the processes that maintain local species diversity of ectomycorrhizal fungi. Plant and Soil 170: 63–73.

Dentinger BTM, Didukh MY, Moncalvo JM. 2011. Comparing COI and ITS barcode markers for mushrooms and allies (*Agaricomycotina*). PLoS One 6(9): e25081. http://dx.doi.org/10.1371/journal.pone.0025081

Gardes M, Bruns TD. 1993. ITS primers with enhanced specificity for Basidiomycetes: application to the identification of mycorrhizae and rusts. Mol. Ecol. 2: 113–118.

Hongo T. 1981. Notulae mycologicae (17). Mem. Fac. Educ. Shiga Univ. Nat. Sci. 31: 33–36.

Horak E. 1987. *Agaricales* from Yunnan, China, I. Trans. Myc. Soc. Japan 28: 171–188.

Kuo M. 2006. *Callistosporium luteo-olivaceum*. Retrieved from the MushroomExpert.Com website: http://www.mushroomexpert.com/callistosporium_luteo-olivaceum.html.

Manimohan P, Leelavathy KM. 1989. Some agarics new to India. Sydowia 41: 200–208.

Munsell ™. 1975. Soil color charts. Baltimore.

Qamar FM, Ali H, Ashraf S, Daud A, Gillani H, Mirza H, Rehman HU. 2011. Distribution and habitat mapping of key fauna species in selected areas of Western Himalaya, Pakistan. Journal of Animal and Plant Sciences 21(2S): 396–399.

Redhead SA. 1982. The systematics of *Callistosporium luteo-olivaceum*. Sydowia 35: 223–235.

Redhead SA. 1997. Macrofungi of British Columbia: requirements for inventory. Res. Br., B.C. Min. For., and Wildl. Br., B.C. Min. Environ., Lands & Parks, Victoria, B.C. Working Paper 28/1997. 119 p.

Singer R. 1970. *Omphalinae* (*Clitocybeae – Tricholomataceae, Basidiomycetes*). Fl. Neotrop. Monogr. 3: 3–84.

Tamura K, Peterson D, Peterson N, Stecher G, Nei M, Kumar S. 2011. Molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. Mol. Biol. Evol. 28(10): 2731–2739.

Wikramanayake E, Dinerstein E, Loucks CJ, Olson DM, Morrison J, Lamoureux J, McKnight M, Hedao P. 2002. Terrestrial ecoregions of the Indo-Pacific: a conservation assessment. Island Press, Washington DC. 643 p.

White TJ, Bruns T, Lee S, Taylor JW. 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. 315–322, in: MA Innis et al. (eds). PCR Protocols: a guide to methods and applications. Academic, New York.

ISSN (print) 0093-4666 © 2014. Mycotaxon, Ltd. ISSN (online) 2154-8889

MYCOTAXON

http://dx.doi.org/10.5248/129.79

Volume 129(1), pp. 79–83 *July–September 2014*

Gastroboletus thibetanus: a new species from China

Shu-Rong Wang[1A], Qi Wang[2], De-Li Wang[1], & Yu Li[1B]*

[1] *Institute of Grassland Science, Northeast Normal University, and Key Laboratory of Vegetation Ecology, Ministry of Education, Changchun, 130024, China*

[2] *Engineering Research Center of Chinese Ministry of Education for Edible and Medicinal Fungi, Jilin Agricultural University, 2888 Xincheng Street, Changchun, 130118, China*

* Correspondence to: [A]*wzlj2005@163.com;* [B]*yuli966@126.com*

Abstract— *Gastroboletus thibetanus* sp. nov. is reported from Tibet, China. It was collected under *Abies forrestii* var. *smithii* and is characterized by pale green-yellow to coral red basidiomata, a percurrent columella, and relatively large basidiospores.

Key words— *Basidiomycota*, *Boletaceae*, *Boletales*, macrofungi, taxonomy

Introduction

Gastroboletus Lohwag (*Boletaceae, Boletales, Basidiomycota*) is a small genus that is widely distributed in temperate regions (Kirk et al. 2008). It is characterized by hypogeous, sequestrate *Boletus*-like basidiomata that have a poorly developed or absent stipe with irregularly arranged tubes (usually covered by persistent membranes) and elliptical to spindle-shaped, smooth, brown to golden brown spores. Spore dispersal depends primarily on animal mycophagy (Nouhra et al. 2002). *Gastroboletus* has been reported from North and South America, Asia, South Africa, Western Europe, and eastern Australia (Cázares & Trappe 1991; Horak 1977; Lohwag 1926; Nouhra & Castellano 1995; Nouhra et al. 2002; Singer & Smith 1964; Smith & Singer 1959; Trappe & Castellano 2000; Thiers & Trappe 1969; Thiers 1979, 1989; Zang 1995). Thiers (1989) divided *Gastroboletus* sensu lato into several genera to reflect their phylogeny more correctly. Nouhra et al. (2002) provided a comprehensive key to all known *Gastroboletus* species.

Mt. Sejila, which is located in Tibet, China, is geographically isolated due to its high altitude and poor road access. Its fungal flora has been poorly studied, with only a few *Cortinarius* species previously reported (Teng et al. 2011). In an attempt to help rectify this, macrofungal field surveys were carried out in 2012

Figure 1. *Gastroboletus thibetanus* (HMJAU 30001, holotype). A. basidioma; B. vertical section of basidioma, showing gleba and columella. Scale bars = 2 cm.

and 2013. Here we describe a particularly interesting hypogeous sequestrate species, which we propose as *G. thibetanus* sp. nov.

Materials & methods

Specimens were collected at Mt Sejila, Tibet, under *Abies forrestii* var. *smithii*, the dominant species in the coniferous forest with *Larix* and *Picea* and with *Rhododendron* occasionally found in the understory (Xu 1995). The acidic brown soil has a pH of 4.9. All specimens are deposited in the Herbarium of Mycology of Jilin Agricultural University (HMJAU), China. Macroscopic characters were described based on fresh and dried material. Color names follow Kornerup & Wanscher (1978). For light microscopic observations, free-hand sections of the specimens were mounted in 5% KOH solution on glass slides. Forty randomly selected basidiospores were measured under 1000× magnification. The basidiospore surfaces were observed by scanning electron microscope after coating with gold (Bozzolla & Russell 1999).

Taxonomic description

Gastroboletus thibetanus Shu R. Wang & Yu Li, **sp. nov.** Figs 1, 2

MycoBank MB 807041

Differs from *Gastroboletus ruber* and *G. molinae* by its larger basidiospores and the absence of cystidia.

Type: China. Tibet, Lenzhi, Mount Sejila, 29°40′22.54″N 94°43′11.20″E, on soil under *Abies forrestii* var. *smithii* Viguié & Gaussen, 26 July 2012, Wang Shu-Rong T10020 (**Holotype**, HMJAU 30001).

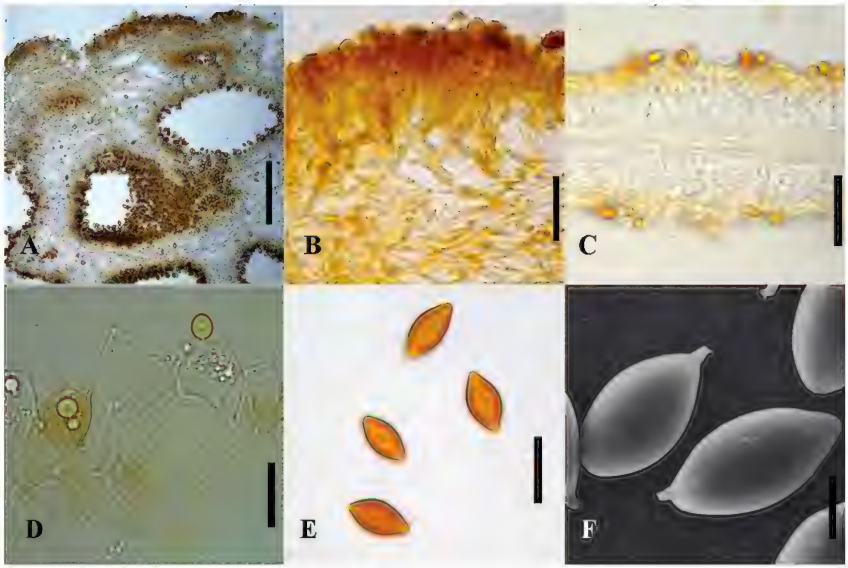


FIGURE 2. *Gastroboletus thibetanus*. A. vertical section through the peridium and gleba of a basidioma; B. peridial structure; C. parallel hyphae in trama; D. basidia and basidiospores; E. basidiospores, showing yellowish color and thick walls; F. SEM micrograph of basidiospores, showing smooth surface. Scale bars: A = 100 μm; B, C = 50 μm; D, E = 20 μm; F = 5 μm.

ETYMOLOGY: Referring to Tibet Autonomous Region, where the holotype was collected.

BASIDIOMATA depressed-globose to reniform, 6–40 mm across when dried, 10–48 mm across when fresh, surface smooth, sometimes cracked-areolate on top, "light green-yellow" to "coral red" (1A7–1A8, 9A7–9B7), becoming somewhat grayish yellow with age. GLEBA 5–23 mm broad, olive brown (4E5), with empty chambers and a prominent columella, changing bluish on bruising or exposure. COLUMELLA 7–15 mm broad, yellow to reddish-brown (2A8, 4A8) when fresh, sordid yellowish when dried, truncate to dendroid, with narrow, radiating branches, extended below as a reddish-brown sterile base, usually arising from yellowish rhizomorphs, changing bluish when bruised or exposed. Odor fruity to mushroomy.

PERIDIUM 60–150 μm thick, yellow-brown. PERIDIAL HYPHAE 3–18 μm diam., surface smooth, thin-walled, yellowish, more or less appressed, sometimes interwoven, slightly gelatinous to floccose in KOH. HYPHAE BELOW PERIDIUM 2.0-4.5 μm diam., surface smooth, yellow-brown, thin-walled, loose and subparallel with the peridium. TRAMA olive brown, consisting of loose, subparallel hyphae (2.0–5.0 μm thick). COLUMELLAR HYPHAE 3.0–6.0 μm diam., thick-walled, pale yellow to pale brown, loose and rarely dichotomously branched. BASIDIA cylindrical, 2–4-spored, 25–36 × 10–14 μm; sterigmata

3.7–5 µm long and 1.5–3 µm thicker at base. Basidiospores ellipsoid, smooth, thick-walled, yellowish in KOH, 11–23.5 × 7.5–12 µm, L/W ratio = 1.64–1.94, Average = 1.80 ± 0.02, symmetrical, basal apiculus prominent. Cystidia and clamp connections not seen.

Habitat: Epigeous or subhypogeous on soil under *Abies forrestii* var. *smithii*.

Additional specimens examined: **China**. **Tibet**, Lenzhi, Mount Sejila, 29°40′22.54″N 94°43′11.20″E, 11 August 2013, coll. Wang Shu-Rong T50457 (HMJAU 30002); 29°40′22.64″N 94°43′10.22″E, 11 August 2013, coll. Wang Shu-Rong T50467 (HMJAU 30005); 29°40′22.50″N 94°43′10.44″E, 4 October 2013, coll. Li Shu T18269 (HMJAU 30006).

Discussion

The combined characters of the pale green-yellow to coral red basidiomata that become bluish on bruising or exposure, percurrent columella, relatively large (11–23.5 × 7.5–12 µm) thick-walled basidiospores, and the absence of cystidia are diagnostic for *G. thibetanus*. *Gastroboletus thibetanus* belongs to *G.* sect. *Gastrosubtomentosi* based on the red overtones of the basidiomata, dry peridial surface, and structure of the peridium (Thiers 1989).

The color and size of *G. thibetanus* basidiomata are somewhat similar to those of *G. ruber* and *G. molinae* reported from U.S.A. (Cázares & Trappe 1991; Nouhra et al. 2002). The two American species differ from *G. thibetanus* by smaller basidiospores (8–20 × 4–6 µm and 11–20 × 5–8 µm, respectively) and the presence of cystidia. In addition, *G. ruber* always occurs in coniferous forests dominated by *Tsuga* spp. (Zeller 1939, Molina et al. 1992) while *G. molinae* is associated with *Quercus chrysolepis* (Nouhra et al. 2002).

Gastroboletus thibetanus also resembles *G. citrinobrunneus, G. dinoffii,* and *G. doii* by tissues that turn blue on bruising or exposure. Both *G. citrinobrunneus* and *G. dinoffii*, reported from California, U.S.A, are readily distinguished by their larger basidiomata and smaller basidiospores (Nouhra & Castellano 1995; Thiers 1979). *Gastroboletus doii* (from New Caledonia) can be differentiated by pale dull brown to ochre basidiomata, presence of cystidia, and a volva (Zang 1995). The type species of *Gastroboletus, G. boedijnii* from China (Lohwag 1926), is easily separated from *G. thibetanus* by the presence of cystidia and smaller (11–14 × 4–5.5 µm) basidiospores (Smith & Singer 1959).

Acknowledgments

We express our deep appreciation to Dr. M. A. Castellano (USDA, Forest Service, Northern Research Station, Corvallis, Oregon, U.S.A) and Dr. Ian R. Hall (Truffles & Mushrooms (Consulting) Ltd., Dunedin, New Zealand), who critically reviewed the manuscript and provided invaluable suggestions. The first author is very grateful to Dr. M. Kakishima (Jilin Agricultural University, China) and Dr. E. Nagasawa (The Tottori Mycological Institute, Japan) for their improvement of the manuscript. This work was

supported by the Fungal Diversity Investigation and Key Technology in Sustainable Use of Rare Fungi in Tibet fund (No. 2012BAC01B04).

Literature cited

Bozzolla JJ, Russell LD. 1999. Electron microscopy: principles and techniques for biologists. 2nd ed. Jones & Bartlett Learning.

Cázares E, Trappe JM. 1991. Alpine and subalpine fungi of the Cascade Mountains. 3. *Gastroboletus ruber* comb. nov. Mycotaxon 42: 339–345.

Horak E. 1977. New and rare boletes from Chile. Bol. Soc. Argentina Bot. 18: 97–109.

Kirk PM, Cannon PF, Minter DW, Stalpers JA. 2008. Ainsworth & Bisby's dictionary of the fungi, 10th edn. Wallingford, CAB International.

Kornerup A, Wanscher JH. 1978. Methuen handbook of colour. Eyre Methuen, London.

Lohwag H. 1926. Zur Entwicklungsgeschichte und Morphologie der Gastromyceten. Beihefte zum Botanischen Centralblatt, 2. Abt., 42: 117–334.

Molina R, Massicotte H, Trappe JM. 1992. Specificity phenomena in mycorrhizal symbioses: community-ecological consequences and practical implications. 357–423, in: MF Allen (ed.). Mycorrhizal functioning: an integrative plant-fungal process. Chapman & Hall, New York.

Nouhra E, Castellano MA. 1995. NATS truffle and truffle-like fungi 3: *Gastroboletus dinoffii* sp. nov. Mycotaxon 55: 179–183.

Nouhra E, Castellano MA, Trappe JM. 2002. NATS truffle and truffle-like fungi 9: *Gastroboletus molinai* [sic] sp. nov. (*Boletaceae, Basidiomycota*), with a revised key to the species of *Gastroboletus*. Mycotaxon 83: 409–414.

Singer R, Smith AH. 1964. Studies on secotiaceous fungi X. Additional data on *Gastroboletus*. Mycologia 56: 310–313. http://dx.doi.org/10.2307/3756548

Smith AH, Singer R. 1959. Studies on secotiaceous fungi. IV. *Gastroboletus, Truncocolumella,* and *Chamonixia*. Brittonia 11: 205–223. http://dx.doi.org/10.2307/2805006

Teng LJ, Xu AS, Liu XJ. 2011. The resources of *Cortinarius* in Sejila Mountain of Mountain of Tibet. Edible Fungi of China 5: 002.

Thiers HD. 1979. New and interesting hypogeous and secotioid fungi from California. Beihefte zur Sydowia 8: 381–390.

Thiers HD. 1989. *Gastroboletus* revisited. Memoirs of the New York Botanical Garden 49: 355–359.

Thiers HD, Trappe JM. 1969. Studies in the genus *Gastroboletus*. Brittonia 21: 244–254. http://dx.doi.org/10.2307/2805576

Trappe JM, Castellano MA. 2000. New sequestrate *Ascomycota* and *Basidiomycota* covered by the Northwest Forest Plan. Mycotaxon 75: 153–179.

Xu FX. 1995. Study on the forest ecosystem in Tibet. Shenyang. Liaoning University Press. [in Chinese].

Zang M. 1995. A new species of the genus *Gastroboletus* from New Caledonia. Mycotaxon 54: 407–412.

Zeller SM. 1939. New and noteworthy gasteromycetes. Mycologia 31: 1–32. http://dx.doi.org/10.2307/3754429

ISSN (print) 0093-4666 © 2014. Mycotaxon, Ltd. ISSN (online) 2154-8889

MYCOTAXON

http://dx.doi.org/10.5248/129.85

Volume 129(1), pp. 85–95 *July–September 2014*

Biogeographical patterns in pyrenomycetous fungi and their taxonomy. 4. *Hypoxylon* and the southern United States

Larissa N. Vasilyeva[1*] & Steven L. Stephenson[2]

[1]*Institute of Biology and Soil Science, Far East Branch of the Russian Academy of Sciences, Vladivostok 690022, Russia*

[2]*Department of Biological Sciences, University of Arkansas, Fayetteville, Arkansas 72701, USA*

* Correspondence to: *vasilyeva@biosoil.ru*

Abstract — A biogeographic region with a peculiar species composition and located in the southern states of the United States is discussed. It is proposed that this region is a part of a possible Caribbean center of fungal biodiversity. *Hypoxylon confertisilvae*, *H. ilicinum*, *H. meridionale*, *H. minicroceum*, and *H. rolingii* are described as species new to science.

Key words — *Ascomycota*, distribution, *Xylariaceae*

Introduction

A group of the southern states of the United States (Arkansas, Alabama, Florida, Louisiana, Mississippi, Missouri, and Texas) represent a region of considerable interest with respect to the biogeography of pyrenomycetous fungi. First, one can observe typical species that are known only from southeastern North America, including such examples as *Biscogniauxia atropunctata* (Schwein.) Pouzar and *Diatrype atlantica* Lar.N. Vassiljeva on *Quercus* spp., *Diatrype tremellophora* Ellis on *Magnolia* spp., and *Diatrype virescens* (Schwein.) M.A. Curtis on *Fagus grandifolia*. Second, there are species that occur mainly in the southern states listed above, with one example being *Camillea signata* (S.C. Jong & C.R. Benj.) Læssøe et al. The same preference for this region is observed in *Biscogniauxia schweinitzii* Y.M. Ju & J.D. Rogers, *Rosellinia glandiformis* Ellis & Everh., and *R. langloisii* Ellis & Everh.

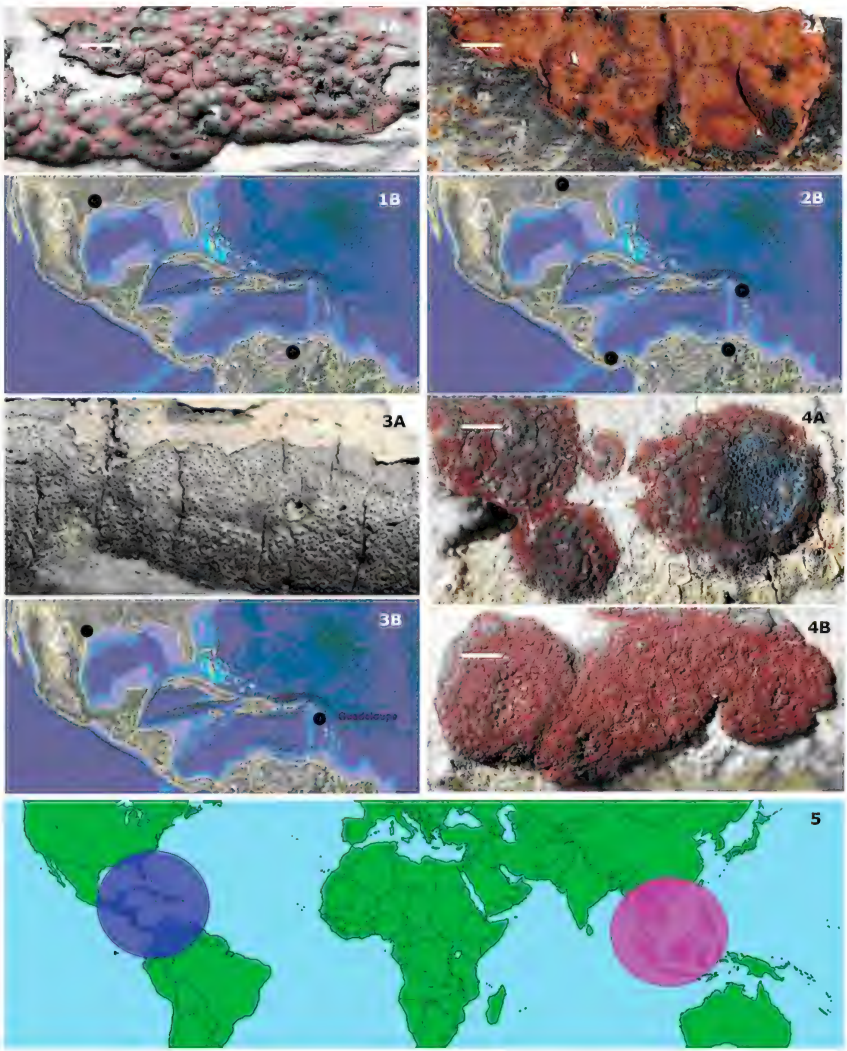
Over a period of several years (2006–11) surveys for pyrenomycetous fungi were carried out in a number of localities in this region, mostly in Arkansas (the Buffalo National River Park, the Ouachita Mountains Biological Station, and the Ozark National Science Center) and Texas (the Big Thicket National Preserve). The survey results combined with literature data suggest a very specific center of fungal biodiversity.

The southern United States as part of a possible Caribbean center of fungal biodiversity

Some species reported for Mexican border regions—*Biscogniauxia arima* F. San Martín et al., *Hypoxylon lividipigmentum* F. San Martín et al., and *Annulohypoxylon thouarsianum* var. *macrosporum* (F. San Martín et al.) Y.M. Ju et al. also have been found in Texas (Big Thicket National Preserve). Several species have distributions that extend from the southern United States to northern South America. Thus, *Hypoxylon venezuelense* Y.M. Ju & J.D. Rogers, described originally from Venezuela (Ju & Rogers 1996), also has been found in Texas (Fig. 1). *Hypoxylon rickii* Y.M. Ju & J.D. Rogers is known from French Guiana, Mexico, and St. John (U.S. Virgin Islands) as well as from Louisiana (Fig. 2). Other Caribbean islands seem to share species of pyrenomycetous fungi with adjacent continental areas, such as *Vivantia guadalupensis* J.D. Rogers et al., described originally from Guadeloupe (Rogers et al. 1996) and later found in Texas (Fig. 3).

There are additional data from some other biogeographic comparisons that support an independent Caribbean center of fungal biodiversity. We have previously discussed the "Asa Gray disjunction," a well-known distribution pattern that exists for many organisms, including fungi (Vasilyeva & Stephenson 2010). This pattern, which is characteristic of species occurring both in northeastern North America and northeastern Asia, is represented by 'sibling' species that replace each other in these two widely separated world regions.

Unexpectedly, a southern parallel to the "Asa Gray disjunction" was found to exist between southeastern Asia (tentatively consisting of the Indochina Peninsula, southern provinces of China, and the Malay Archipelago) and the region in and around the Caribbean Sea and the Gulf of Mexico (Fig. 5). When efforts are made to identify species collected in the Indo-Malayan center of biodiversity (Vasilyeva et al. 2012), the other species they most closely resemble often appear to be those reported from Mexico, the southern United States, or the Caribbean coast of South America. Sometimes, the morphological resemblance is so high that the species from the eastern and western hemispheres differ in only one or two features. (The same phenomenon is observed for the species involved in the northern "Asa Gray disjunction.") For example, *Biscogniauxia schweinitzii* from the southern United States and *B. lithocarpi* Lar.N. Vassiljeva et al. from northern Thailand have strikingly similar asci and ascospores but differ in their stromata. *Hypoxylon rubroargillaceum* Lar.N. Vassiljeva et al. is similar to *H. flavoargillaceum* J.H. Mill. (known from Colombia and Venezuela) but differs in ascal tips that do not turn blue in Melzer's reagent and the presence of granules immediately beneath the surface and among the perithecia that are not yellowish-brown but bright-red.



Figs. 1–5. 1. ***Hypoxylon venezuelense:*** A. Stroma (VLA P–2605), B. Collection localities; 2. ***Hypoxylon rickii:*** A. Stroma (VLA P–2607), B. Collection localities; 3. ***Vivantia guadalupensis:*** A. Stroma (VLA P–2448), B. Collection localities; 4A–B. Stromata of ***Hypoxylon subchlorinum*** (VLA P–2604); 5. Tentative location of the Caribbean and Indo-Malayan centers of fungal diversity. Scale bars: 1A = 1.5 mm; 2A = 9 mm; 3A = 4.5 mm; 4A–B = 2.5 mm. [The maps modified to show our data are taken from the web sites <http://www.biomedcentral.com> (Figs 1B, 2B, 3B) and <http://www.designus.com/vector-world-map> (Fig. 5).]

Such morphological similarities shown by species associated with eastern and western centers of biodiversity is not surprising, given that molecular studies also pull together exactly such species from those areas. Thus, the most interesting and relevant xylariaceous phylogenetic tree (Hsieh et al. 2005) clusters together several species (*Hypoxylon crocopeplum* Berk. & M.A. Curtis, *H. dieckmannii* Theiss., *H. erythrostroma* J.H. Mill., *H. fendleri* Cooke, and *H. haematostroma* Mont.) from both Mexico (the Caribbean center) and Taiwan (the Indo-Malayan center). While this might support conspecificity of specimens from the western and eastern hemispheres, the specimens could just as easily be assigned to different species, as has been done for *Hypoxylon lividicolor* Y.M. Ju & J.D. Rogers from Taiwan and *H. lividipigmentum* from Mexico, two taxa that also cluster together with the same level of support (Hsieh et al. 2005) and which we have already (Vasilyeva & Stephenson 2010) suggested might represent a vicarious pair of species. The pantropical distribution of a number of species as well as the species concepts themselves might have to be reconsidered in the light of the existence of more restricted biogeographic centers of biodiversity.

Supporting data from non-pyrenomycetous fungi

The peculiar species composition of the fungal assemblages found in the southern United States and the occurrence of many species in only some portions of the Caribbean have been emphasized by basidiomycete specialists. For some of these, Wu & Mueller (1997) described a "Florida-Texas distribution pattern." The extensive literature on fleshy basidiomycetes described from Texas (Lewis & Ovrebo 2009) lists a number of species known only from Texas [e.g., *Boletus lewisii* (Both) Bessette et al., *Clitocybe texensis* H.E. Bigelow, *Cortinarius lewisii* O.K. Mill., *Megacollybia texensis* R.H. Petersen & D.P. Lewis, *Russula lewisii* Buyck, *R. texensis* Buyck et al.] or species more widely distributed in the southern United States bordering the Caribbean Sea. Thus, *Phylloporus boletinoides* A.H. Sm. & Thiers is known from Alabama, Florida, and Texas (Singer et al. 1990), whereas *Amanita westii* (Murrill) Murrill has been reported from Florida, Mississippi and Texas (Tulloss & Lewis 1994).

More recent information on basidiomycetes restricted to countries adjacent to the Caribbean Sea has been published. For example, *Ganoderma ravenelii* Steyaert is known from the southeastern states and Mexico (Torres-Torres & Guzmán-Dávalos 2008). Guzmán-Dávalos (2002) indicated that only 13% of Mexican *Gymnopilus* species are distributed worldwide, whereas the remaining 87% occur in only Mexico, in the United States and Mexico, in the United States, Mexico, and Cuba, or in Mexico and Central America. A number of agarics and boletes occur primarily in the montane oak forests of Costa Rica and Colombia (Halling & Mueller 2002). Lodge et al. (2002) note that "49 species of *Hygrocybe*,

Camarophyllopsis and *Cuphophyllus* are found in southeastern United States, excluding species in common with the Lesser Antilles, Trinidad and Venezuela" and that "58.8% (10/17) of the 38 southern Caribbean species would eventually be found in the Greater Antilles." Here the reference to "Caribbean species" surely indicates the existence of a special center of fungal biodiversity.

There are even some data on vicarious species in the Caribbean and Indo-Malayan centers of diversity. As such, *Trogia venenata* Zhu L. Yang et al. was described recently from southern China counterbalanced by a closely related Neotropical species [*Trogia buccinalis* (Mont.) Pat.], described originally from Guyana (Yang et al. 2012).

Possible resurrection of some species

At present, the names of some species reported from the southeastern United States have been reduced to synonyms, but their biogeographic distributional pattern suggests a separate taxonomic identity. For example, *Hypoxylon epiphaeum* Berk. & M.A. Curtis, which is restricted to *Magnolia* spp., was reported [as a variety of *H. investiens* (Schwein.) M.A. Curtis] for Alabama, Florida, Georgia, Louisiana, New Jersey, New York, North Carolina, South Carolina, and West Virginia (Miller 1961). The species also has been found twice in Texas (both on *Magnolia virginiana* L. from the David Lewis property near Bleakwood in Newton Co. and the Turkey Creek Unit of the Big Thicket National Preserve), but *H. epiphaeum* is treated by Ju & Rogers (1996) as a synonym of *H. monticulosum* Mont., a fungus commonly reported from the tropics and subtropics on different kinds of wood. Granted, the two species share some similarities (e.g., papillate ostioles, ascospore size, perispore dehiscent in 10% KOH). One Big Thicket specimen lacked KOH-extractable pigments and so resembled *H. monticulosum*, while another (seemingly younger) specimen displayed dark-livid to dark-vinaceous pigments. We differentiate *H. epiphaeum* from *H. monticulosum* by the possession of glomerate stromata with an underlying orange-red subiculum (Fig. 6) and purple pigments in the young stages and its association with *Magnolia* species in the southeastern United States. Unfortunately, the holotype of "*H. epiphaeum*" in Kew has stromata characteristic of *H. monticulosum,* leaving the fungus associated with *Magnolia* without a proper name so that a new name should be proposed for a common species.

Another example is *Hypoxylon subchlorinum* Ellis & Calk. (Fig. 4), described originally from Florida and also found in Georgia and Texas. Ju & Rogers (1996, p. 124) cite the name *H. subchlorinum* under *H. fuscum* (Pers.) Fr., although they admit, "additional study might further subdivide this species." They accept a rather wide ascospore length range (8-20 µm) for *H. fuscum,* whereas northern temperate specimens usually have ascospores 12–15 µm long

(Miller 1961). *Hypoxylon subchlorinum* has smaller ascospores and differently shaped stromata. There is also a discrepancy between the original description of *H. subchlorinum* and its type specimens. Ellis & Everhart (1888) described the stromata as yellow and the ascospores as 7–8 µm long. However, the ascospores are 8–10.5 µm long in both holotype and one isotype of *H. subchlorinum* (both parts of exsiccate North American Fungi 2115: Florida, Jacksonville, 1886, Calkins, W.W., corticated wood). A piece of the same exsiccate studied in Herbarium of Michigan University was characterized by the flattened and rounded stromata also observed in the material from the Big Thicket. The Texas material was primarily from *Carpinus virginiana* Mill., but very similar stromata were found in a specimen of *H. subchlorinum* on *Celtis* from Louisiana (Farlow Herbarium: St. Martinville, 28 Jan 1889, A.B. Langlois). This species, which seems to be a characteristic representative of Florida, Louisiana, and Texas should be segregated from the "*H. fuscum*" complex.

A third candidate in need of resurrection is *Hypoxylon mulleri* J.H. Mill., described originally from Puerto Rico (Miller 1933) and later found in Florida (Miller 1961) and Texas (Big Thicket National Preserve). Its name has been suggested as synonymous with *H. placentiforme* Berk. & M.A. Curtis, but based on material collected in Texas, the shiny black stromata with conspicuous perithecial mounds of *H. mulleri* differ from the brown vinaceous and very smooth stromata of *H. placentiforme.* The *H. placentiforme* concept of Ju & Rogers (1996), which accepts 8.5–18.5 µm long ascospores, seems rather broad; such a range is very unusual, given the nature of repetitive variability within the *Hypoxylon* (cf. Vasilyeva & Stephenson 2010: Table 2). It would appear that the range has been 'synthesized' from several different taxonomic entities, and indeed, the synonyms cited under *H. placentiforme* have been applied to taxa with narrower ascospore ranges (Miller 1961, Whalley & Taligoola 1978). Prominent examples are *H. sclerophaeum* var. *macrosporum* (ascospores 14–20 µm long) and *H. sclerophaeum* var. *microsporum* (ascospores 7.5–12 µm long), whereas the lectotype of *H. placentiforme* [K(M)125651] from Cuba has ascospores 11–15 µm long.

Taxonomy

Hypoxylon confertisilvae Lar.N. Vassiljeva & S.L. Stephenson, **sp. nov.** Fig. 6C

MycoBank MB 808385

Differs from *Hypoxylon fuscum* by its finely papillate stromatal surface and KOH-extractable pigments between cinnamon and umber.

Type—USA, Texas, Big Thicket National Preserve, Turkey Creek Unit, Kirby Nature Trail, on bark of *Acer* sp., 4 Apr 2011, L. Vasilyeva (VLA P-2601).

Etymology—refers to the "thick forest" implying the place of collection (Big Thicket National Preserve)

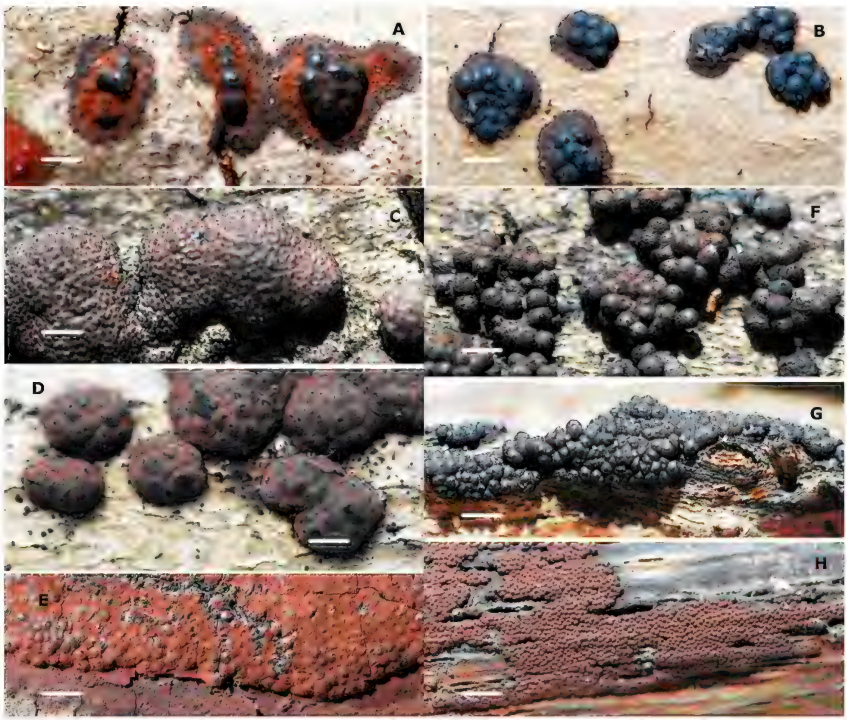


Fig. 6. Stromata: A–B. "***Hypoxylon epiphaeum***" (VLA P-2454); C. ***Hypoxylon confertisilvae;*** D. ***Hypoxylon ilicinum;*** E. ***Hypoxylon meridionale;*** F. ***Hypoxylon croceum*** from the Great Smoky Mountains National Park (VLA P–1617); G. ***Hypoxylon minicroceum;*** H. ***Hypoxylon rolingii.*** Scale bars: A–B = 1.5 mm; C = 1.7 mm; D = 2.4 mm; E = 4.5 mm; F = 1 mm; G = 1.7 mm; H = 2.4 mm.

Stromata hemispherical to pulvinate, discrete 2–4 mm diam., with finely conspicuous perithecial mounds; surface dark-vinaceous; tissue black immediately beneath surface and among the perithecia, with KOH-extractable pigments between cinnamon (62) and umber (9). Perithecia 100–150 µm diam., ostioles black and finely papillate. Asci in spore-bearing part 85–90 × 6–7 µm, stalks 30–40 µm, with apical ring bluing in Melzer's iodine reagent, discoid, 0.3–0.5 µm high, 2.5–3 µm broad. Ascospores light brown, unicellular, elongated-ellipsoid, almost equilateral, 12–15 × 4.5–5 µm, germ slit not observed, perispore indehiscent in 10% KOH.

Comments—This species is similar to *Hypoxylon fuscum* in stromatal shape and color but differs with respect to the stromatal surface and KOH-extractable pigments. It also is similar to *H. minicroceum* (see below) in ascospore shape and color and the KOH-extractable pigments but differs in the stromatal shape and the larger ascospores.

Hypoxylon ilicinum Lar.N. Vassiljeva & S.L. Stephenson, **sp. nov.** FIG. 6D

MYCOBANK MB 808386

Differs from *Hypoxylon fuscum* by its smaller ascospores and luteous KOH-extractable pigments.

TYPE—USA, Texas, Big Thicket National Preserve, Jack Gore Baygall Unit, Blue Hole Trail, on branches of *Ilex* sp., 11 Apr 2011, L. Vasilyeva (VLA P-2593).

ETYMOLOGY—refers to the association with branches of *Ilex* sp.

STROMATA hemispherical to pulvinate, discrete 2–4 mm diam. or confluent, with inconspicuous or slightly conspicuous perithecial mounds; surface livid-vinaceous to dark-vinaceous; light brownish granules immediately beneath surface and among the perithecia, with KOH-extractable pigments luteous. PERITHECIA spherical, 200–300 µm diam., ostioles umbilicate. ASCI in the spore-bearing part 75–80 × 6–7 µm, stalks 35–40 µm, with an apical ring bluing in Melzer's iodine reagent, discoid, 0.3–0.5 µm high, 2.5–3 µm broad. ASCOSPORES brown, unicellular, ellipsoid-inequilateral, 8–12.5 × 4.5–5.5 µm, with a straight germ slit extending the length of the spore on the concave side; perispore dehiscent in 10% KOH.

COMMENTS—This species resembles *H. fuscum* in shape and color of the stromata but differs in having smaller ascospores and KOH-extractable pigments. It also is similar to *H. porphyreum* Granmo in general appearance (Granmo 1999: Fig. 31) and ascospore size, but pigments of *H. porphyreum* are said to be "brown with a greenish tone" in 10% KOH. Moreover, *H. porphyreum* is found only on *Quercus* in Norway and Sweden. Another similar species is *H. vinosopulvinatum* Y.M. Ju et al. from Taiwan (Ju et al. 2004), which has pulvinate stromata of the same color, similar KOH-extractable pigments, comparably sized ascospores, and a perispore dehiscent in 10% KOH. However, H. *vinosopulvinatum* has rather conspicuous perithecial mounds and a germ slit on the convex side of the ascospore. The two species—*H. ilicinum* and *H. vinosopulvinatum*—could be considered vicarious, replacing each other in the Caribbean and Indo-Malayan centers of fungal biodiversity.

Hypoxylon meridionale Lar.N. Vassiljeva & S.L. Stephenson, **sp. nov.** FIG. 6E

MYCOBANK MB 808387

Differs from *Hypoxylon dieckmannii* by its larger and darker, as well as strongly inequilateral, ascospores.

TYPE—USA, Texas, Big Thicket National Preserve, Big Sandy Creek Unit, Woodlands Trail, on wood, 3 Apr 2011, L. Vasilyeva (VLA P-2602).

ETYMOLOGY—refers to the south (of the United States).

STROMATA widely effused, with slightly conspicuous perithecial mounds, surface brown vinaceous; cream-whitish granules immediately beneath surface, with grayish sepia (108) pigments. PERITHECIA spherical, 200–300 µm

diam., ostioles umbilicate. Asci not observed. Ascospores brown, ellipsoid-inequilateral, 8–12(12.5) × 4–4.5 µm, with a slightly oblique germ slit less than the length of the spore, perispore indehiscent in 10% KOH.

Comments—The species is similar to *H. dieckmannii*, which can also possess brown vinaceous stromata with grayish sepia KOH-extractable pigments, 6.5–10(11) µm long ascospores, and a perispore indehiscent in 10% KOH. *Hypoxylon dieckmannii* has been reported from Brazil, French Guiana, Mexico, and Louisiana (New Orleans) in the United States, so its occurrence in Texas could be expected. However, the specimen from the Big Sandy Creek Unit—with a similar stromatal surface, pigments, and indehiscent perispore—has longer (although overlapping) ascospores (10–12 µm) that do not fall into the characteristic ascospore range for *H. dieckmannii*. In addition, Miller (1961: 33, Fig. 48) described *H. dieckmannii* as having 6–8 µm long ascospores and illustrated these as small and light brown; their shape (almost equilateral and ellipsoid) also differs from the ascospores of Texas specimens, which are darker and strongly inequilateral. It also should be noted that the fresh stromata produced an olivaceous pigment in NaOH, a feature that suggests *H. anthochroum* Berk. & Broome, but *H. meridionale* differs from *H. anthochroum* in having an indehiscent perispore.

Hypoxylon minicroceum Lar.N. Vassiljeva & S.L. Stephenson, **sp. nov.** Fig. 6G

MycoBank MB 808388

Differs from *Hypoxylon croceum* by its smaller stromata and KOH-extractable pigments.

Type—USA, Texas, Big Thicket National Preserve, Jack Gore Baygall Unit, Blue Hole Trail, rotten wood, 11 Apr 2011, L. Vasilyeva (VLA P-2591).

Etymology—refers to the similarity with *Hypoxylon croceum*.

Stromata glomerate to effuse-pulvinate, with conspicuous to almost naked perithecial mounds; surface fuscous; dark brown woody tissue immediately beneath surface and among the perithecia, with KOH-extractable pigments between umber (9) and cinnamon (62). Perithecia spherical, (100–)120–150 µm diam., ostioles papillate. Asci in spore-bearing part 80–95 × 4.5–5 µm, stalks 17–20 µm, with apical ring bluing in Melzer's iodine reagent, discoid, 0.5–0.7 µm high, 1.5–2 µm broad. Ascospores light brown, unicellular, narrow-ellipsoid, almost equilateral, 10–12.5 × 4–4.5 µm, with a straight germ slit the length of the spore; perispore indehiscent in 10% KOH.

Comments—The stromata of *H. minicroceum* resemble those of *H. croceum* J.H. Mill. (Fig. 6F) but are much smaller. Both species have comparable ascospore size and a perispore indehiscent in 10% KOH, but they differ in their KOH-extractable pigments. *Hypoxylon croceum* has been reported from a restricted area in the southeastern United States (Georgia, North

Carolina, Ohio, and Tennessee) and also has been reported from Venezuela (Ju & Rogers 1996). When the perithecial mounds are not very prominent, *H. minicroceum* is similar to *H. submonticulosum* Y.M. Ju & J.D. Rogers but differs in the cinnamon KOH-extractable pigments and ascospore shape. With respect to its small and inconspicuous stromata, *H. minicroceum* is similar to *H. inconspicuum* J.D. Rogers & Y.M. Ju, described originally from Costa Rica (Ju et al. 2005), which differs in a number of features (smaller ascospores, dark vinaceous KOH-extractable pigments and umbilicate ostioles).

Hypoxylon rolingii Lar.N. Vassiljeva & S.L. Stephenson, **sp. nov.** FIG. 6H

MycoBank MB 808389

Differs from *Hypoxylon crocopeplum* by its very thin stromata of a different color and the KOH-extractable pigments.

TYPE—USA, Texas, Big Thicket National Preserve, large palmetto area along Little Pine Island Bayou, on dead branches, 8 Apr 2011, L. Vasilyeva (VLA P-2590).

ETYMOLOGY—after the volunteer Paul Roling, who provided assistance to researchers in the Big Thicket National Preserve.

STROMATA effused-pulvinate, with conspicuous perithecial mounds, very thin (about 0.15–0.2 mm); surface bay (6) or dark-vinaceous (82), with dark tissue immediately beneath surface and among perithecia, with fresh KOH-extractable pigments chestnut (40) or sepia (63), but umber (9) or cinnamon (62) after the slides dry up. PERITHECIA spherical, 150–200 µm diam., ostioles black and very faintly papillate. ASCI in the spore-bearing part 70–75 × 5–6 µm, stalks 25–30 µm long, with an apical ring bluing in Melzer's iodine reagent, discoid, 0.5–0.7 µm high, 1.5–2 µm broad. ASCOSPORES light brown, unicellular, ellipsoid, almost equilateral, 10–13.5 × 4–5 µm, with a straight germ slit extending the length of the spore; perispore indehiscent in 10% KOH.

COMMENTS—The stromata are very thin but are somewhat reminiscent of those in *H. crocopeplum* in having rather conspicuous perithecia mounds. In many other respects (e.g., color of surface and KOH-extractable pigments, indehiscent perispore), *H. rolingii* differs from *H. crocopeplum*.

Acknowledgments

We express our thanks to the personnel of the Big Thicket National Preserve, Buffalo National River Park, Ouachita Mountains Biological Station, and the Ozark National Science Center for providing an opportunity to collect at these localities. We also thank Dr. Santiago Chacón (Instituto de Ecología, México) and David Lewis (Newton, TX) for serving as presubmission reviewers.

Literature cited

Ellis JB, Everhart BM. 1888. Synopsis of the North American species of *Hypoxylon* and *Nummularia*. Journal of Mycology 4: 85–93. http://dx.doi.org/10.2307/3752760

Granmo A. 1999. Morphotaxonomy and chorology of the genus *Hypoxylon* (*Xylariaceae*) in Norway. Sommerfeltia 26: 1–81.

Guzmán-Dávalos L. 2002. Tropical brown- and black-spored Mexican agarics with particular reference to *Gymnopilus*. 61–71, in: R Watling et al. (eds). Tropical Mycology: Vol. 1. Macromycetes. CABI Publishing, Wallingford.

Halling RE, Mueller GM. 2002. Agarics and boletes of Neotropical oakwoods. 1–10, in: R Watling et al. (eds). Tropical Mycology: Vol. 1. Macromycetes. CABI Publishing, Wallingford.

Hsieh JM, Ju YM, Rogers JD. 2005. Molecular phylogeny of *Hypoxylon* and closely related genera. Mycologia 97: 844–865. http://dx.doi.org/10.3852/mycologia.97.4.844

Ju YM, Rogers JD. 1996. A revision of the genus *Hypoxylon*. Mycologia Memoir 20: 1–365.

Ju YM, Rogers JD, Hsieh HM. 2004. New *Hypoxylon* species and notes on some names associated with or related to *Hypoxylon*. Mycologia 96: 154–161. http://dx.doi.org/10.2307/3761997

Ju YM, Rogers JD, Hsieh HM. 2005. New *Hypoxylon* and *Nemania* species from Costa Rica and Taiwan. Mycologia 97: 562–567. http://dx.doi.org/10.3852/mycologia.97.2.562

Lewis DP, Ovrebo CL. 2009. Mycological literature on Texas fleshy Basidiomycota, two new combinations, and new fungal records for Texas. Journal of the Botanical Research Institute of Texas 3: 257–271.

Lodge DJ, Baroni TJ, Cantrell SA. 2002. Basidiomycetes of the Greater Antilles project. 45–60, in: R Watling et al. (eds). Tropical Mycology: Vol. 1. Macromycetes. CABI Publishing, Wallingford.

Miller JH. 1933. Some new species of *Hypoxylon*. Mycologia 25: 321–329. http://dx.doi.org/10.2307/3754100

Miller JH. 1961. A monograph of the world species of *Hypoxylon*. University of Georgia Press, Athens. 158 p.

Rogers JD, Ju YM, Candoussau F. 1996. *Biscogniauxia anceps* comb. nov. and *Vivantia guadalupensis* gen. et sp. nov. Mycological Research 100: 669–674. http://dx.doi.org/10.1016/S0953-7562(96)80196-1

Singer R, Ovrebo CL, Halling RE. 1990. New Species of *Phylloporus* and *Tricholomopsis* from Colombia, with notes on *Phylloporus boletinoides*. Mycologia 82: 452–459. http://dx.doi.org/10.2307/3760016

Torres-Torres MG, Guzmán-Dávalos L. 2008. Taxonomic status and new localities for *Ganoderma ravenelii*. Mycotaxon 103: 33–40.

Tulloss RE, Lewis DP. 1994. *Amanita westii* – taxonomy and distribution. A rare species from states bordering the Gulf of Mexico. Mycotaxon 50: 131–138.

Vasilyeva LN, Stephenson SL. 2010. Biogeographical patterns in pyrenomycetous fungi and their taxonomy. 1. The Grayan disjunction. Mycotaxon 114: 281–303. http://dx.doi.org/10.5248/114.281

Vasilyeva LN, Stephenson SL, Hyde KD, Bahkali AH. 2012. Some stromatic pyrenomycetous fungi from northern Thailand – 1. *Biscogniauxia*, *Camillea* and *Hypoxylon* (*Xylariaceae*). Fungal Diversity 55: 65–76. http://dx.doi.org/10.1007/s13225-011-0150-9

Whalley AJS, Taligoola HK. 1978. Species of *Hypoxylon* from Uganda. Transactions of the Botanical Society of Edinburgh 42: 93–98. http://dx.doi.org/10.1080/03746607808685328

Wu QX, Mueller GM. 1997. Biogeographic relationships between the macrofungi of temperate eastern Asia and eastern North America. Canadian Journal of Botany 75: 2108–2116. http://dx.doi.org/10.1139/b97-922

Yang ZL, Li YC, Shi GQ, Zeng G. 2012. *Trogia venenata* (*Agaricales*), a novel poisonous species which has caused hundreds of deaths in southwestern China. Mycological Progress 11: 937–945. http://dx.doi.org/10.1007/s11557-012-0809-y

ISSN (print) 0093-4666 © 2014. Mycotaxon, Ltd. ISSN (online) 2154-8889

MYCOTAXON

http://dx.doi.org/10.5248/129.97

Volume 129(1), pp. 97–108 *July–September 2014*

Rhizophagus natalensis, a new species in the *Glomeromycota*

Janusz Błaszkowski[1*], Gerard Chwat[1], Anna Góralska[1] & Bruno T. Goto[2]

[1]*Department of Ecology and Protection of Environment, West Pomeranian University of Technology in Szczecin, Szczecin, Słowackiego 17, PL-71434 Szczecin, Poland*

[2]*Departamento de Botânica, Ecologia e Zoologia, CB, Universidade Federal do Rio Grande do Norte, Campus Universitário, 59072-970, Natal, RN, Brazil*

[*]Correspondence to: *janusz.blaszkowski@zut.edu.pl*

Abstract — Morphological and histochemical studies of spores and phylogenetic analyses of LSU and SSU nrDNA sequences have indicated that a fungal species found in maritime dunes of the Parque Estadual das Dunas de Natal "Journalista Luiz Maria Alves" located in Natal, Brazil, represents an undescribed species of *Rhizophagus*. The new species, *R. natalensis*, forms spores singly or (sometimes) in loose clusters in soil. Its spores are pastel yellow to light yellow and globose to subglobose [(75-)101(-133) µm diam] or (rarely) ovoid (50-70 × 63-79 µm) and have one subtending hypha with a pore that is open or occluded by a septum. Its spore wall comprises four layers: (1) a semi-permanent hyaline layer forming the spore surface, (2) a permanent hyaline unit layer, (3) a laminate pastel yellow to light yellow layer, and (4) a flexible hyaline layer. Layers 1 and 3 stain in Melzer's reagent.

Key words — arbuscular mycorrhizal fungi, molecular phylogeny, tropical ecosystem

Introduction

Arbuscular mycorrhizal fungi (AMF) of the phylum *Glomeromycota* live in symbiosis with ca. 70-90% of land plants growing in different habitats (Smith & Read 2008, Brundrett 2009). Sand dunes are especially favorable sites for development of AMF, mainly due to the sparse populations of other microorganisms competing for nutrients produced by living plants and the rare occurrence of AMF microparasites (Błaszkowski 1994, Dalpé 1989, Koske 1987, Lee & Koske 1994, Tadych & Błaszkowski 2000). On the other hand, the existence of plants growing in extremely poor and harsh dune sites may well depend highly on the establishment of such symbioses, in that AMF frequently increase the supply of nutrients to plants and decrease their sensitivity to

different abiotic and biotic stresses (Bothe et al. 2010, Schönbeck 1978, Dehn & Schüepp 1989, Griffioen & Ernst 1989, Smith & Read 2008).

The approximately 250 AMF species that have been described (Schüßler & Walker 2010) probably represent less than 5% of world's AMF species (Krüger et al. 2009). Of the described species ca. 62% produce glomoid spores, i.e., spores similar in mode of formation, spore wall structure, and subtending hyphal characters to those of *Glomus macrocarpum* Tul. & C. Tul. (type species of the genus *Glomus;* Clements & Shear 1931).

Glomoid spores are produced by AMF of the genus *Rhizophagus* P.A. Dang. in the family *Glomeraceae* Piroz. & Dalpé (Redecker et al. 2013; Schüßler & Walker 2010). Long after Dangeard (1896) described *Rhizophagus,* Gerdemann & Trappe (1974) synonymized the genus with *Glomus*. Recently, however, Schüßler & Walker (2010) resurrected *Rhizophagus* based on both the original description of its type species, *R. populinus* P.A. Dang., stating that the fungus produced spores abundantly inside roots, and the phylogenetic sequence analyses of *Rhizophagus* and other AMF that tend to form intraradical spores.

We studied the morphological and histochemical characters of glomoid spores extracted from a pot trap culture inoculated with rhizosphere soil and root fragments of an unrecognized grass colonizing maritime sand dunes of the Parque Estadual das Dunas de Natal "Journalista Luiz Maria Alves" located in Natal, Brazil. Our research suggested that we had found an undescribed species similar to *R. fasciculatus* (Thaxt.) C. Walker & A. Schüssler. Later phylogenies inferred from analyses of sequences of the large (LSU) and small (SSU) subunit genes support our original hypothesis that the two fungi represent different species. The Brazilian fungus is described below as *R. natalensis.*

Materials & methods

Establishment and growth of trap and single-species cultures, extraction of spores, and staining of mycorrhiza

Spores examined in this study derived from a pot trap culture. The trap culture was established to obtain living spores and to initiate sporulation of specimens that may not have sporulated in the field collections (Stutz & Morton 1996). Methods used to establish the trap culture, growth conditions, and spore extraction follow Błaszkowski et al. (2012). The growing substrate of the trap culture was the field-collected rhizosphere soil and roots of the plant species sampled mixed with autoclaved coarse-grained sand (1:1 v/v).

Eight single-species cultures were also established and grown as given in Błaszkowski et al. (2012). The single cultures were set up with ca. 10 spores and small clusters of spores (5–12) attached by a common mycelium. Unfortunately, all cultures failed.

Microscopy and nomenclature

Morphological features of spores and their wall structure were determined after examining at least 100 spores mounted in water, lactic acid, polyvinyl alcohol/lactic

acid/glycerol (PVLG; Omar et al. 1979), and a mixture of PVLG and Melzer's reagent (1:1, v/v). Glomerospores at all developmental stages were mounted in PVLG and PVLG+Melzer's reagent, then covered with a cover slip, crushed to varying degrees by applying pressure to the cover slip, stored at 65°C for 24 h to clear their contents from oil droplets, and examined under an Olympus BX 50 compound microscope equipped with Nomarski differential interference contrast optics. Microphotographs were recorded on a Sony 3CCD color video camera coupled to the microscope.

Spore structure terminology follows Stürmer & Morton (1997) and Walker (1983). Spore color was examined under a dissecting microscope on fresh specimens immersed in water. Color names are from Kornerup & Wanscher (1983). Nomenclature of fungi and the authors of fungal names are from Index Fungorum (http://www.indexfungorum.org/AuthorsOfFungalNames.htm). Voucher specimens were mounted in PVLG and a mixture of PVLG and Melzer's reagent (1:1, v/v) on slides and deposited in the Department of Ecology and Protection of Environment (DEPE), West Pomeranian University of Technology in Szczecin, Szczecin, Poland, and in the herbarium at Universidade Federal do Rio Grande do Norte (UFRN) in Natal, Rio Grande do Norte, Brazil.

DNA extraction, polymerase chain reaction and DNA sequencing

Crude DNA was isolated from 3–8 single spores crushed with a needle in ultra clean water on sterile microscope slides under a dissecting microscope. Amplification, cloning, and sequencing procedures followed Błaszkowski et al. (2012). The large subunit nrDNA gene (partial) of the fungus newly described here was amplified using the primers LR1 (Tuinen et al. 1998) and FLR2 (Trouvelot et al. 1999) and then the primers 28G1 and 28G2 (Silva et al. 2006), and SSU nrDNA gene (partial) was amplified with the primers AML1 and AML2 (Lee et al. 2008) as described in Błaszkowski et al. (2012). The segment spanning SSU (partial), internal transcribed region (ITS1, 5.8S and ITS2, full), and LSU (partial) nrDNA of *Septoglomus africanum* (Błaszk. & Kovács) Sieverd. et al., *S. furcatum* Błaszk. et al., *S. fuscum* Błaszk. et al., and *S. xanthium* (Błaszk. et al.) G.A. Silva et al. were amplified using a nested procedure and the SSUmAf-LSUmAr primer pair for the first nested PCR and the SSUmCf-LSUmBr primer pair for the second nested PCR, as suggested by Krüger et al. (2009). Representatives of sequences have been deposited in GenBank (KJ210823–KJ210828).

Sequence alignment and phylogenetic analyses

The glomeromycotan origin of the sequences was initially tested by BLAST (Zhang et al. 2000) search. To determine the generic affiliation of our new species we performed pilot phylogenetic analyses separately with all LSU and SSU sequences and those representing all recognized glomeromycotan genera with glomoid spores available in GenBank and published by Krüger et al. (2012). The results of the SSU sequence analyses are not presented here. The final data set used to generate the LSU tree in Fig. 9 comprised six sequences from our new species, three sequences each from known *Rhizophagus* spp. and one to three sequences, published or obtained by us, from 12 other species in the *Glomeraceae*. Sequences representing *Claroideoglomus claroideum* (N.C. Schenck & G.S. Sm.) C. Walker & A. Schüssler served as outgroup in all analyses. The LSU and SSU sequences were aligned with PRANK$_{F+}$ with the $_{F+}$ option invoked

to fix already inferred indels at their place and avoid another indel being inferred in an overlapping position during the second recursion of the algorithm (Löytynoja & Goldman 2008). Overhanging sequence fragments of both termini were trimmed. Bayesian (BI) analyses were performed with MrBayes 3.1 (Huelsenbeck & Ronquist 2001, Ronquist & Huelsenbeck 2003), and maximum likelihood (ML) analyses with PHYML (Guindon & Gascuel 2003). Before the analyses the best-fit substitution models for the alignments were estimated by the Akaike information criterion (AIC) using TOPALi v. 2.5 (Milne et al. 2004). In the BI analyses of both LSU and SSU sequences the model employed was GTR + G, and TrN + G was applied in the ML analyses of both types of sequences. In the BI analyses the Markov chain was run for 5,000,000 generations, sampling in every 500 steps with a burn-in at 3000. In the ML analyses the transition/transversion ratio for DNA models and the gamma distribution parameter were estimated. Six substitution rate categories were set. Topology and branch lengths and rate parameters were optimized. Support of branches in the ML analyses was estimated in bootstrap analyses with 1000 replicates. The details of the analyses are available on request. Phylogenetic trees were visualized and edited in MEGA5 (Tamura et al. 2011).

Taxonomy

Rhizophagus natalensis Błaszk., Chwat & B.T. Goto, **sp. nov.** Figs 1–8

MycoBank MB 808282

Differs from *Rhizophagus irregularis* in spore color and shape and the number and phenotypic and histochemical characters of the spore wall layers.

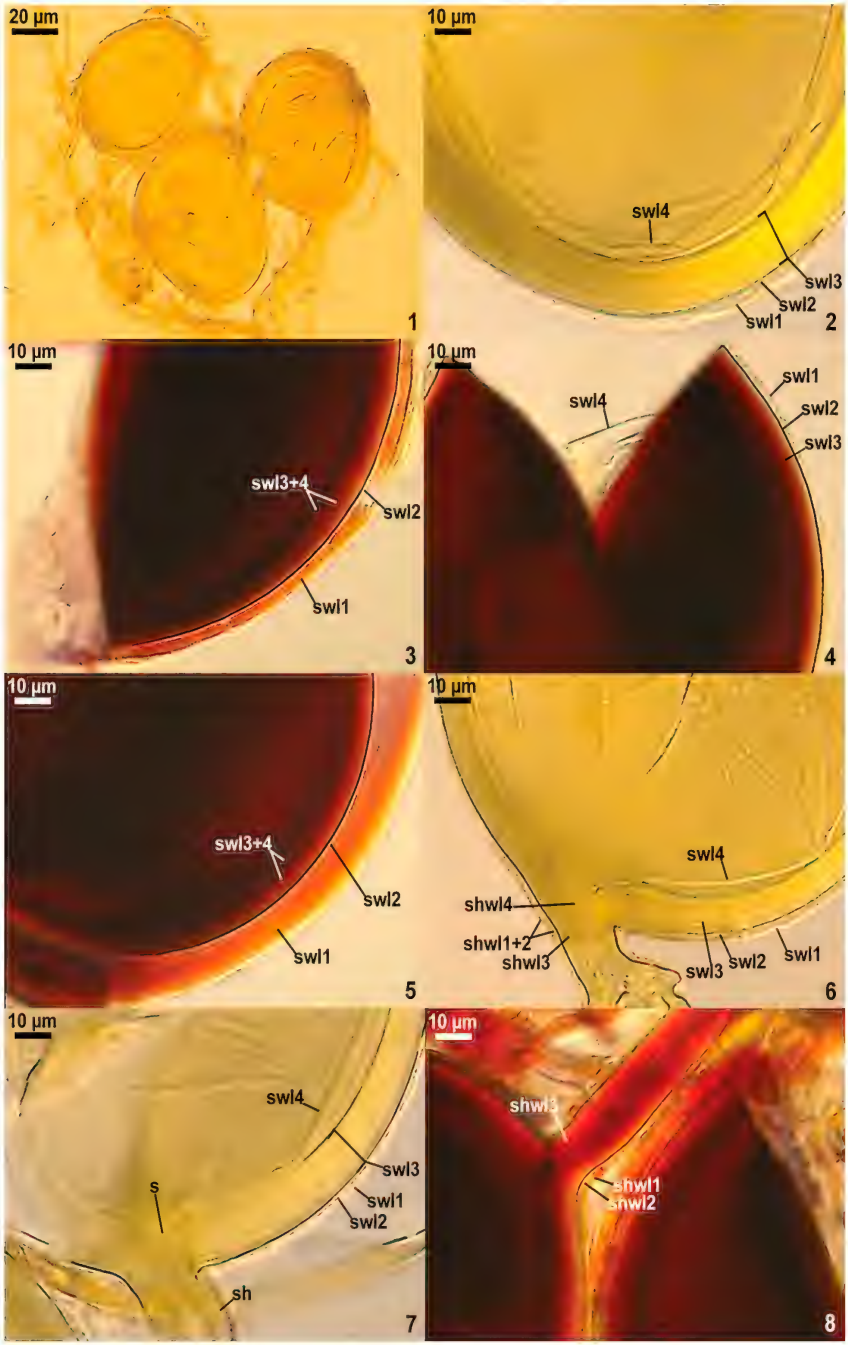
Type: Brazil, Rio Grande do Norte, Natal, Parque Estadual das Dunas de Natal "Journalista Luiz Maria Alves", under an unrecognized grass colonizing maritime sand dunes, 12 Sept. 2012, J. Błaszkowski 3381 (**Holotype**, DEPE; GenBank KJ210823), J. Błaszkowski 3382–3384 (**Isotypes**, DEPE; GenBank KJ210824–KJ210828).

Etymology. Latin, *natalensis*, referring to the 'Natal' dunes from which the species was collected.

Sporocarps unknown.

Glomerospores formed singly, sometimes in loose clusters, in soil; developing blastically at the tip of sporogenous hyphae either directly from mycorrhizal extraradical hyphae (single spores) or branched from a parent hypha continuous with a mycorrhizal extraradical hypha (spores in clusters). Clusters 160–989 × 180–1200 µm with 2–14 spores. Spores pastel yellow (3A4) to light yellow (3A5); globose to subglobose; (75–)101(–133) µm diam; rarely ovoid; 50–70 × 63–79 µm; with one subtending hypha.

Figs. 1–8. *Rhizophagus natalensis* spores (differential interference microscopy). **1**. Spores in loose cluster. **2–5**. Spore wall layers (swl) 1–4; note the unstained layer 4 in Fig. 4 and the swollen layer 1 in Fig. 5. **6**. Spore wall layers (swl) 1–4 continuous with subtending hyphal wall layers (shwl) 1–4; note shwl4 extending along the inner surface of shwl3. **7**. Spore wall layers (swl) 1–4, of which layer 4 forms a curved septum (s) in the subtending hyphal (sh) lumen. **8**. Subtending hyphal wall layers (shwl) 1–3 continuous with spore wall layers 1–3; shwl1 and swl1 are not visible. Figs 1, 2, 6, 7, in PVLG; Figs 3–5, 8, in PVLG+Melzer's reagent.


20 µm
1
10 µm
swl4
swl3
swl2
swl1
2
10 µm
swl3+4
swl2
swl1
3
10 µm
swl4
swl1
swl2
swl3
4
10 µm
swl3+4
swl2
swl1
5
10 µm
swl4
shwl4
shwl1+2
shwl3
swl3
swl2
swl1
6
10 µm
swl4
swl3
s
swl1
swl2
sh
7
10 µm
shwl3
shwl1
shwl2
8

Spore wall consists of four layers: layer 1, forming the spore surface, semi-permanent, hyaline, (1.0–)2.4(–5.3) µm thick, slowly deteriorating with age, usually present as a more or less decomposed structure even in older spores; sometimes swelling and becoming up to 8.3 µm thick; layer 2, a unit layer, permanent, smooth, hyaline, (0.8–)1.1(–1.5) µm thick; layer 3 laminate, smooth, pastel yellow (3A4) to light yellow (3A5), (6.3–)8.7(–14.0) µm thick, consisting of laminae up to 0.8–1.0 µm thick, frequently easily separating from each other in crushed spores; and layer 4 flexible, smooth, hyaline, (0.8–)1.3(–2.0) µm thick, usually separating from the lower surface of layer 3 in crushed spores. Layers 1 and 3 stain reddish white (9A2) to greyish red (10C5) and brownish violet (11D8) to violet brown (11E8) in Melzer's reagent, respectively.

Subtending hypha pastel yellow (3A4) to light yellow (3A5); straight or recurved, cylindrical to funnel-shaped, sometimes slightly constricted at the spore base; (16.0–)20.8(–29.8) µm wide at the spore base. Wall of subtending hypha pale pastel yellow (3A4) to light yellow (3A5); (7.3–)9.4(–14.3) µm thick at the spore base; continuous with spore wall layers 1–4. Pore (1.5–)2.4(–3.3) µm diam, open when spore wall layer 4 develops from subtending hyphal wall layer 4 arising far below the spore base and extending along the inner surface of subtending hyphal wall layer 3 or occluded by a curved septum continuous with spore wall layer 4 and open or occluded from the reasons mentioned above and gradually narrowing due to thickening of wall layer 3 of the subtending hypha towards the centre of its lumen.

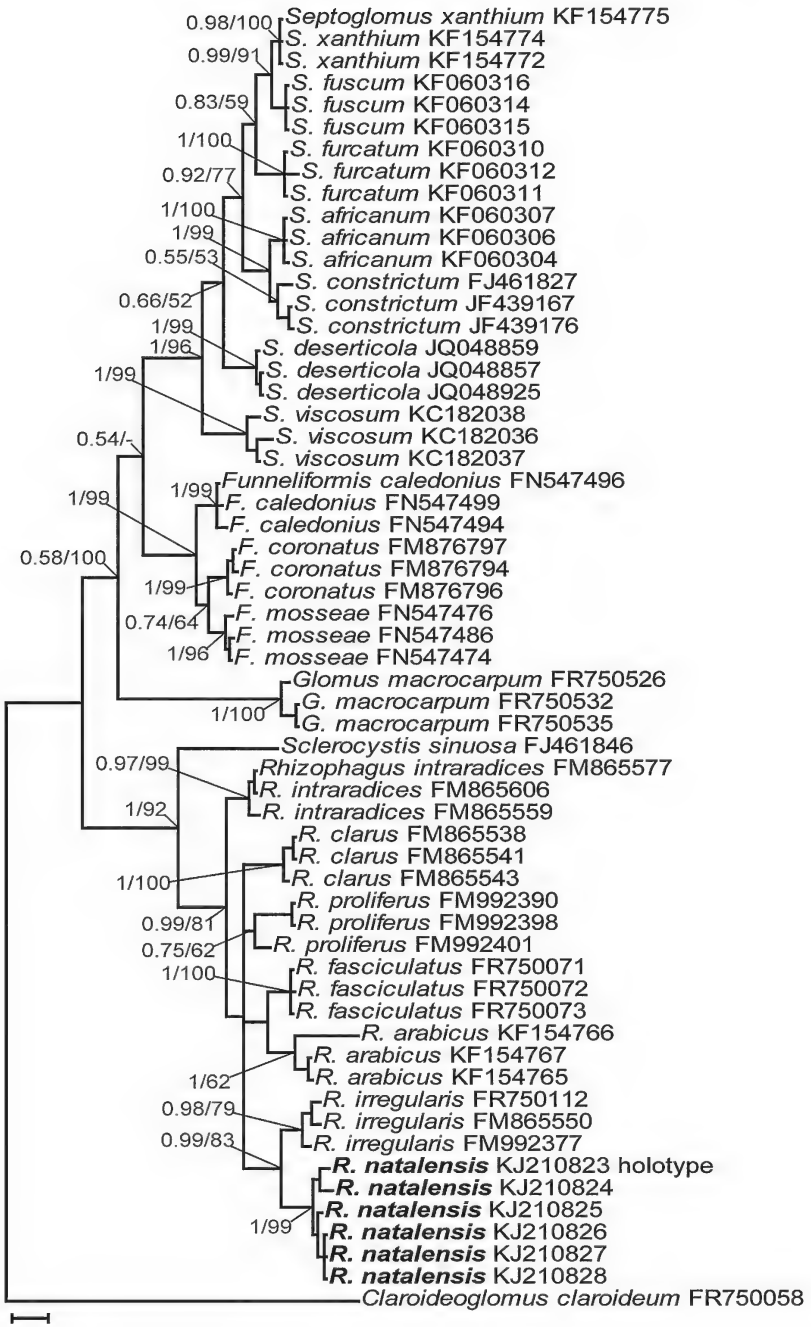
Germination unknown.

Mycorrhizal associations — In the field *R. natalensis* was associated with roots of an unrecognized plant species of *Poaceae* that colonized the Atlantic Ocean sand dunes in the Parque Estadual das Dunas de Natal "Journalista Luiz Maria Alves" (5°46′S, 35°12′W), one of the largest urban conservation areas with dune vegetation in Brazil. In a trap culture inoculated with the rhizosphere soil and root fragments of the plant, the fungus lived in symbiosis with *Plantago lanceolata* and sporulated abundantly. However, *R. natalensis* stopped producing spores after ca. two years of growing of this culture. All attempts to establish single-species cultures from spores extracted from the trap culture failed.

Phylogenetic position — In BI and NJ trees with LSU sequences the *R. natalensis* clade obtained high values of statistical support and was sister to

---

Fig. 9. 50% majority rule consensus phylogram of AMF species inferred from a Bayesian analysis of LSU nrDNA sequences of our new species and 33 other species, including *Claroideoglomus claroideum* as outgroup. The new species is shown in boldface. The fungal names are followed by GenBank accession numbers. The Bayesian posterior probabilities ≥0.50 and ML and NJ bootstrap values ≥50% are shown near the branches, respectively. Bar indicates 0.5 expected change per site per branch.


0.98/100
Septoglomus xanthium KF154775
S. xanthium KF154774
0.99/91
S. xanthium KF154772
S. fuscum KF060316
0.83/59
S. fuscum KF060314
S. fuscum KF060315
1/100
S. furcatum KF060310
0.92/77
S. furcatum KF060312
S. furcatum KF060311
1/100
S. africanum KF060307
1/99
S. africanum KF060306
0.55/53
S. africanum KF060304
S. constrictum FJ461827
0.66/52
S. constrictum JF439167
1/99
S. constrictum JF439176
1/96
S. deserticola JQ048859
1/99
S. deserticola JQ048857
S. deserticola JQ048925
S. viscosum KC182038
0.54/-
S. viscosum KC182036
S. viscosum KC182037
1/99
Funneliformis caledonius FN547496
1/99
F. caledonius FN547499
F. caledonius FN547494
0.58/100
F. coronatus FM876797
F. coronatus FM876794
1/99
F. coronatus FM876796
F. mosseae FN547476
0.74/64
F. mosseae FN547486
1/96
F. mosseae FN547474
Glomus macrocarpum FR750526
1/100
G. macrocarpum FR750532
G. macrocarpum FR750535
Sclerocystis sinuosa FJ461846
0.97/99
Rhizophagus intraradices FM865577
R. intraradices FM865606
1/92
R. intraradices FM865559
R. clarus FM865538
R. clarus FM865541
1/100
R. clarus FM865543
R. proliferus FM992390
0.99/81
R. proliferus FM992398
0.75/62
R. proliferus FM992401
1/100
R. fasciculatus FR750071
R. fasciculatus FR750072
R. fasciculatus FR750073
R. arabicus KF154766
R. arabicus KF154767
1/62
R. arabicus KF154765
R. irregularis FR750112
0.98/79
R. irregularis FM865550
0.99/83
R. irregularis FM992377
R. natalensis KJ210823 holotype
R. natalensis KJ210824
R. natalensis KJ210825
1/99
R. natalensis KJ210826
R. natalensis KJ210827
R. natalensis KJ210828
Claroideoglomus claroideum FR750058

a clade with *R. irregularis* (Błaszk. et al.) C. Walker & A. Schüssler (Fig. 9). In BI and ML trees with SSU sequences *R. natalensis* was strongly supported as monophyletic and sister to a clade comprising *R. fasciculatus*, *R. intraradices* (N.C. Schenck & G.S. Sm.) C. Walker & A. Schüssler, *R. irregularis*, and *R. vesiculiferus* (Thaxt.) C. Walker & A. Schüssler distributed into two subclades (data not shown).

Distribution & habitat — Spores of *R. natalensis* were found only in one pot trap culture inoculated with a mixture of the rhizosphere soil and root fragments of an unrecognized plant species of *Poaceae* growing in maritime sand dunes of the Parque Estadual das Dunas de Natal "Journalista Luiz Maria Alves" located in Natal, Brazil. Data on the climate, vegetation and soil chemical properties of the dunes in which *R. natalensis* occurred were described previously (Goto et al. 2012).

Spores of *R. natalensis* were not found in either ca. 3000 field-collected soils and ca. 3000 pot trap cultures representing different regions of Europe, Africa, Asia, Cuba, and the U.S.A. (Błaszkowski, pers. observ.).

However, BLAST queries indicated that the *R. natalensis* LSU sequences we obtained were 99% similar to: (1) five LSU sequences (FR871319, FR871318, FR871325, FR871323, FR871320) of AMF colonizing roots and rhizosphere soils of *Brachypodium retusum* (Pers.) P. Beauv. and *Bromus rubens* L. growing in semiarid degraded area located in the natural ecological park "Vicente Blanes" in Molina de Segura, Province of Murcia, southern Spain (Torrecillas et al. 2012); (2) an LSU sequence (EF066656) of an AMF coming from soil collected under *Medicago* spp. cultivated in a Mediterranean fallow land located at the Mas d'Imbert, France (Pivato et al. 2007); and (3) 55 SSU sequences of uncultured AMF from, e.g., Spain (Alguacil et al. 2012; Torrecillas et al. 2012). This suggests that *R. natalensis* has a wide world distribution.

Discussion

The distinguishing morphological and histochemical characters of *R. natalensis* are its light yellow, relatively large spores with a four-layered spore wall, of which layers 1 and 3 stain intensively in Melzer's reagent. In addition, based on DNA sequence analyses, the species is phylogenetically unique.

Our LSU sequence analyses indicate that *R. natalensis* is most closely related to *R. irregularis* (see "Phylogenetic position" above and Fig. 9). Mature *R. irregularis* spores are frequently hyaline (vs. no mature hyaline spores in *R. natalensis*) and ovoid to oblong or irregular with deep local depressions (Błaszkowski 2012; Błaszkowski et al. 2008; vs. usually globose to subglobose without depressions). The spore wall of *R. irregularis* comprises three (vs. four) layers, of which layer 1 frequently is highly thickened at the spore top and forming a cap-like swell (vs. no such thickening), a laminate innermost layer

(vs. a flexible innermost layer 4), and the only spore wall component (vs. layers 1 and 3) that stains in Melzer's reagent. The subtending hypha of *R. irregularis* spores is 0.6–2.5-fold narrower and has a 1.7–4.9-fold thinner wall at the spore base and its pore may be open or closed by a septum, but the septum forms some innermost laminae of the laminate spore wall layer 3 (vs. spore wall layer 4 in *R. natalensis*). In addition, *R. irregularis* tends to form spores in oblong clusters inside roots and in soil, because the spores arise either terminally from or intercalary inside sporogenous hyphae developing from spore wall layer 1 of an earlier fully differentiated parent spore or/and a branch of the parent hypha continuous with a mycorrhizal extraradical hypha (Błaszkowski 2012; Błaszkowski et al. 2008; vs. no such mode of spore formation in *R. natalensis*).

SSU sequence analyses also support a close relationship of *R. natalensis* with *R. fasciculatus*, *R. intraradices*, and *R. vesiculiferus* (data not shown). Intact *R. natalensis* spores observed under low and high microscope magnifications are almost morphologically and histochemically identical to those of *R. fasciculatus*. Both species form spores singly and in clusters and the spores are similar in color and size and stain intensively in Melzer's reagent (Błaszkowski 2012; Morton, http://invam.wvu.edu/). Examination of spores crushed in PVLG and PVLG+Melzer's reagent readily separates both species. The spore wall of *R. fasciculatus* is 3-layered (lacking layer 2 of the 4-layered wall of *R. natalensis*). Spore wall layer 1 of *R. natalensis*, forming the spore surface, is semi-permanent (vs. permanent in *R. fasciculatus*), may be 2.0–2.9-fold thicker, sometimes swells up to 8.3 µm thick, thereby showing its plasticity (vs. no such behaviour), and may stain more intensively in Melzer's reagent (vs. reddish white; Błaszkowski 2012). In addition, the laminae of the laminate structural spore wall layer 3 of *R. natalensis* are ca. 2-fold thicker than those of the laminate structural spore wall layer 2 of *R. fasciculatus* (<0.5 µm thick) and the *R. natalensis* laminae frequently separate from each other in crushed spores (vs. usually remaining adherent in *R. fasciculatus*). Finally, the subtending hypha of *R. natalensis* spores is less regular in shape (cylindrical to funnel-shaped vs. cylindrical in *R. fasciculatus*), has a 5.2–6.5-fold thicker wall at the spore base, and its pore may be open or occluded by a septum continuous with spore wall layer 4 (vs. closed by a septum continuous with spore wall layer 3).

Freshly mature spores of *R. intraradices* usually are light yellow with a greenish tint (Błaszkowski 2012, Morton http://invam.wvu.edu/, Stürmer & Morton 1997) lacking in *R. natalensis* spores. The spore wall of *R. intraradices* is 1.3–1.8-fold thinner, comprises only three (vs. four) layers of which only the laminate layer 3 (vs. layers 2–4) is permanent, and only spore wall layer 1 (vs. layers 1 and 3) stains in Melzer's reagent. In addition, the subtending hypha of *R. intraradices* spores is less regular in shape (cylindrical to slightly flared vs. cylindrical to funnel-shaped) and 1.2–1.6-fold narrower at the spore base; its

wall is 1.8–2.2-fold thinner at the spore base and its pore is open (vs. open or closed by a septum).

Rhizophagus vesiculiferus is most clearly separated from *R. natalensis* by the formation of sporocarps with a peridium-like layer of thin-walled vesicles (Berch & Fortin 1984, Gerdemann & Trappe 1974). In addition, *R. vesiculiferus* spores are ca. 1.6-fold smaller, their spore wall is 2-layered (vs. 4-layered) and 2.2–2.9-fold thinner, and their subtending hypha is ca. 1.7-fold narrower with an open pore (vs. open or occluded by a septum).

Acknowledgements

The authors acknowledge Dr. Fritz Oehl (Agroscope, Federal Research Institute for Sustainability Sciences, Plant-Soil Interactions, Zürich, Switzerland) and Dr. Gladstone Alves da Silva (Departamento de Micologia, CCB, Universidade Federal de Pernambuco, Av. Prof. Nelson Chaves s/n, Recife, PE, Brazil) for reviewing the manuscript and making helpful suggestions. We also appreciate the corrections and suggestions by Dr. Shaun Pennycook (Nomenclature Editor) and Dr. Lorelei Norvell (Editor-in-Chief). This study was supported in part by Polish National Centre of Science, grants no. 05/B/NZ8/00498 and 07/N/NZ8/02363 and the Fundação de Amparo à Ciência e Tecnologia do Estado do Rio Grande do Norte (FAPERN-PPP) and CASADINHO UFRN/FIOCRUZ (Process N° 552577/2011-1), which provided a financial support to J. Błaszkowski as "visiting professor".

Literature cited

Alguacil MM, Torrecillas E, Roldán A, Díaz G, Torres MP. 2012. Perennial plant species from semiarid gypsum soils support higher AMF diversity in roots than the annual *Bromus rubens*. Soil Biol. Biochem. 49: 132–138. http://dx.doi.org/10.1016/j.soilbio.2012.02.024

Berch SM, Fortin JA. 1984. Some sporocarpic *Endogonaceae* from eastern Canada. Can. J. Bot. 62: 170–180. http://dx.doi.org/10.1139/b84-029

Błaszkowski J. 1994. Arbuscular fungi and mycorrhizae (*Glomales*) of the Hel Peninsula, Poland. Mycorrhiza 5: 71–88. http://dx.doi.org/10.1007/BF00204022

Błaszkowski J. 2012. *Glomeromycota*. W. Szafer Institute of Botany, Polish Academy of Sciences, Kraków.

Błaszkowski J, Czerniawska B, Wubet T, Schäfer T, Buscot F, Renker C. 2008. *Glomus irregulare*, a new arbuscular mycorrhizal fungus in the *Glomeromycota*. Mycotaxon 106: 247–267.

Błaszkowski J, Kovács GM, Gáspár BK, Balázs TK, Buscot F, Ryszka P. 2012. The arbuscular mycorrhizal *Paraglomus majewskii* sp. nov. represents a new distinct basal lineage in *Paraglomeraceae* (*Glomeromycota*). Mycologia 104: 148–156. http://dx.doi.org/10.3852/10-430

Bothe H, Turnau K, Regvar M. 2010. The potential role of arbuscular mycorrhizal fungi in protecting endangered plants and habitats. Mycorrhiza 20: 445–457. http://dx.doi.org/10.1007/s00572-010-0332-4

Brundrett MC. 2009. Mycorrhizal associations and other means of nutrition of vascular plants: understanding the global diversity of host plants by resolving conflicting information and developing reliable means of diagnosis. Plant Soil 320: 37–77. http://dx.doi.org/10.1007/s11104-008-9877-9

Clements FE, Shear CL. 1931. The genera of fungi. Hafner Publishing Co., New York, USA. http://dx.doi.org/10.5962/bhl.title.54501

Dalpé Y. 1989. Inventaire et repartition de la flore endomycorhizienne de dunes et de rivages maritimes du Quebec, du Nouveau-Brunswick et de la Nouvelle-Ecosse. Naturaliste Canadien 116: 219–236.

Dangeard PA. 1896. Une maladie du peuplier dans l'ouest de la France. Le Botaniste 5: 38–43.

Declerck S, Cranenbrouck S, Dalpé Y, Séguin S, Grandmougin-Ferjani A, Fontaine J, Sancholle M. 2000. *Glomus proliferum* sp. nov.: a description based on morphological, biochemical, molecular and monoxenic cultivation data. Mycologia 92: 1178–1187. http://dx.doi.org/10.2307/3761485

Dehn B, Schüepp H. 1989. Influence of VA mycorrhizae on the uptake and distribution of heavy metals in plants. Agric. Ecosys. Environm. 29: 79–83. http://dx.doi.org/10.1016/0167-8809(90)90258-F

Gerdemann JW, Trappe JM. 1974. The *Endogonaceae* in the Pacific Northwest. Myc. Memoir 5: 1–76.

Goto BT, Jardim JG, Silva GA da, Furrazola E, Torres-Arias Y, Oehl F. 2012. *Glomus trufemii* (*Glomeromycetes*), a new sporocarpic species from Brazilian sand dunes. Mycotaxon 120: 1–9. http://dx.doi.org/10.5248/120.1

Griffioen WA, Ernst WHO. 1989. The role of VA mycorrhiza in the heavy metal tolerance of *Agrostis capillaris* L. Agric. Ecosys. Environm. 29: 173–177. http://dx.doi.org/10.1016/0167-8809(90)90258-F

Guindon S, Gascuel O. 2003. A simple, fast, and accurate algorithm to estimate large phylogenies by maximum likelihood. Syst. Biol. 52: 696–704. http://dx.doi.org/10.1080%2F10635150390235520

Huelsenbeck JP, Ronquist F. 2001. MRBAYES: Bayesian inference of phylogeny. Bioinformatics 17: 754–755. http://dx.doi.org/10.1093/bioinformatics/17.8.754

Kornerup A, Wanscher JH. 1983. Methuen handbook of colour. 3rd ed. London: Eyre Methuen.

Koske RE. 1987. Distribution of VA mycorrhizal fungi along a latitudinal temperature gradient. Mycologia 79: 55–68. http://dx.doi.org/10.2307/3807744

Krüger M, Stockinger H, Krüger C, Schüßler A. 2009. DNA-based species level detection of *Glomeromycota*: one PCR primer set for all arbuscular mycorrhizal fungi. New Phytol. 183: 212–223. http://dx.doi.org/10.1111%2Fj.1469-8137.2009.02835.x

Krüger M, Krüger C, Walker C, Stockinger H, Schüßler A. 2012. Phylogenetic reference data for systematics and phylotaxonomy of arbuscular mycorrhizal fungi from phylum to species level. New Phytol. 193: 970–984. http://dx.doi.org/10.1111/j.1469-8137.2011.03962.x

Lee PJ, Koske RE. 1994. *Gigaspora gigantea*: parasitism of spores by fungi and actinomycetes. Mycol. Res. 98: 458–466. http://dx.doi.org/10.1016/S0953-7562(09)81205-7

Lee J, Lee S, Young PW. 2008. Improved PCR primers for the detection and identification of arbuscular mycorrhizal fungi. FEMS Microbiol. Ecol. 65: 339–349. http://dx.doi.org/10.1111/j.1574-6941

Löytynoja A, Goldman N. 2008. An algorithm for progressive multiple alignment of sequences with insertions. Proc. Natl. Acad. Sci. U.S.A. 102: 10557–10562. http://dx.doi.org/10.1073/pnas.0409137102

Milne I, Wright F, Rowe G, Marshal DF, Husmeier D, McGuire G. 2004. TOPALi: Software for automatic identification of recombinant sequences within DNA Multiple Alignments. Bioinformatics 20: 1806–1807. http://dx.doi.org/10.1093/bioinformatics/bth155

Omar MB, Bollan L, Heather WA. 1979. A permanent mounting medium for fungi. Bull. Br. Mycol. Soc. 13: 31–32. http://dx.doi.org/10.1016/S0007-1528(79)80038-3

Pivato B, Mazurier S, Lemanceau P, Siblot S, Berta G, Mougel C, Tuinen D van. 2007. *Medicago* species affect the community composition of arbuscular mycorrhizal fungi associated with roots. New Phytol. 176: 197–210. http://dx.doi.org/0.1111/j.1469-8137.2007.02151.x

Redecker D, Schüßler A, Stockinger H, Stürmer SL, Morton JB, Walker C. 2013. An evidence-based consensus for the classification of arbuscular mycorrhizal fungi (*Glomeromycota*). Mycorrhiza: published online. http://dx.doi.org/10.1007/s00572-013-0486-y

Ronquist F, Huelsenbeck JP. 2003. MRBAYES 3: Bayesian phylogenetic inference under mixed models. Bioinformatics 19: 1572–1574. http://dx.doi.org/10.1093/bioinformatics/btg180

Schönbeck F. 1978. Einfluss der endotrophen Mykorrhiza auf die Krankheitsresistenz höherer Pflanzen. Z. PflKrankh. PflSchutz. 85: 191–196.

Schüßler A, Walker C. 2010. The *Glomeromycota*. A species list with new families and new genera. Schüßler A., Walker C. Gloucester, Published in libraries at Royal Botanic Garden Edinburgh, Kew, Botanische Staatssammlung Munich, and Oregon State University. http://dx.doi.org/10.5248/116.75

Silva GA da, Lumini E, Maia LC, Bonfante P, Bianciotto V. 2006. Phylogenetic analysis of *Glomeromycota* by partial LSU rDNA sequences. Mycorrhiza 16: 183–189. http://dx.doi.org/10.1007/s00572-005-0030-9

Smith SE, Read DJ. 2008. Mycorrhizal symbiosis. 3rd ed. San Diego: Academic Press.

Stürmer SL, Morton JB. 1997. Developmental patterns defining morphological characters in spores of four species in *Glomus*. Mycologia 89: 72–81. http://dx.doi.org/10.5248/116.75

Stutz JC, Morton JB. 1996. Successive pot cultures reveal high species richness of arbuscular mycorrhizal fungi in arid ecosystems. Can. J. Bot. 74: 1883–1889. http://dx.doi.org/10.5248/116.75

Tadych M, Błaszkowski J. 2000. Arbuscular fungi and mycorrhizae (*Glomales*) of the Słowiński National Park, Poland. Mycotaxon 74: 463–483.

Tamura K, Peterson D, Peterson N, Stecher G, Nei M, Kumar S. 2011. MEGA5: molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. Mol. Biol. Evol. 28: 2731–2739. http://dx.doi.org/10.1093/molbev/msr121

Torrecillas E, Alguacil MM, Roldán A. 2012. Differences in the AMF diversity in soil and roots between two annual and perennial gramineous plants co-occurring in a Mediterranean, semiarid degraded area. Plant Soil 354: 97–106. http://dx.doi.org/10.1007/s11104-011-1047-9

Trouvelot S, Tuinen D van, Hijri M, Gianinazzi-Pearson V. 1999. Visualization of ribosomal DNA loci in spore interphasic nuclei of glomalean fungi by fluorescence in situ hybridization. Mycorrhiza 8: 203–206. http://dx.doi.org/10.1007/s005720050235

Tuinen D van, Zhao B, Gianinazzi-Pearson V. 1998. PCR in studies of AM fungi: from primers to application. 387-400, in: AK Varma (ed.). Mycorrhizal manual. Springer, Berlin Heidelberg New York.

Walker C. 1983. Taxonomic concepts in the Endogonaceae: spore wall characteristics in species descriptions. Mycotaxon 18: 443–455.

Zhang Z, Schwartz S, Wagner L, Miller W. 2000. A greedy algorithm for aligning DNA sequences. J. Comput. Biol. 7: 203–14. http://dx.doi.org/10.1089/10665270050081478

ISSN (print) 0093-4666 © 2014. Mycotaxon, Ltd. ISSN (online) 2154-8889

MYCOTAXON

http://dx.doi.org/10.5248/129.109

Volume 129(1), pp. 109–118 *July–September 2014*

A new species of *Nawawia* from Malaysia, with a synopsis of the genus

Teik-Khiang Goh*, Wai-Yip Lau, & Kah-Cheng Teo

Centre for Biodiversity Research, Faculty of Science, Universiti Tunku Abdul Rahman, Bandar Barat, 31900 Kampar, Perak, Malaysia

* Correspondence to: *gohtk@utar.edu.my*

Abstract — *Nawawia quadrisetulata* sp. nov., collected from submerged wood in Malaysia, is described and illustrated. It differs from other *Nawawia* species in having conidia with 4(–5) distal setulae. A synopsis of species in the genus is provided.

Key words — dematiaceous hyphomycete, lignicolous fungi, mitosporic fungi, saprotroph, taxonomy

Introduction

The anamorphic genus *Nawawia* was introduced by Marvanová (1980) based on *Clavatospora filiformis* Nawawi (Nawawi 1973), a hyphomycete found on submerged decaying twigs and petioles. The genus is characterized by having conidiophores that are dematiaceous, thick-walled, septate, percurrently proliferating, and bearing a terminal phialide with distinct collarette and producing unicellular hyaline conidia that are turbinate-tetrahedral to obpyramidal in shape, with their blunt corners at the distal end each provided with a distinct hair-like appendage. There are five reported species of *Nawawia* (Index Fungorum 2014): three from Malaysia (Crous et al. 2009, Kuthubutheen et al. 1992, Nawawi 1973), one from South Africa (Hyde et al. 1996), and one from Russia (Mel'nik & Hyde 2006). Hyde et al. (1996) described *N. dendroidea*, a synnematous species, and compared it with *Chalarodes* McKenzie and *Phialosporostilbe* Mercado & J. Mena. A sporodochial species occurring on leaves, *N. malaysiana*, was described by Crous et al. (2009).

During a survey of fungal diversity from submerged wood in Malaysia, we found an undescribed species of *Nawawia*, which is proposed here as

N. quadrisetulata sp. nov. Its holotype is conserved at the herbarium of the Centre for Biodiversity Research, Faculty of Science, Universiti Tunku Abdul Rahman (Perak campus), Kampar, Malaysia (UTAR). The ultrastructural features of the conidia were studied under the scanning electron microscope (FESEM, Model: JSM-6701F, JEOL, Japan). A synopsis of the six species in the genus is provided.

Taxonomy

Nawawia quadrisetulata Goh, W.Y. Lau & K.C. Teo, **sp. nov.** Figs 1–35

MycoBank MB807053

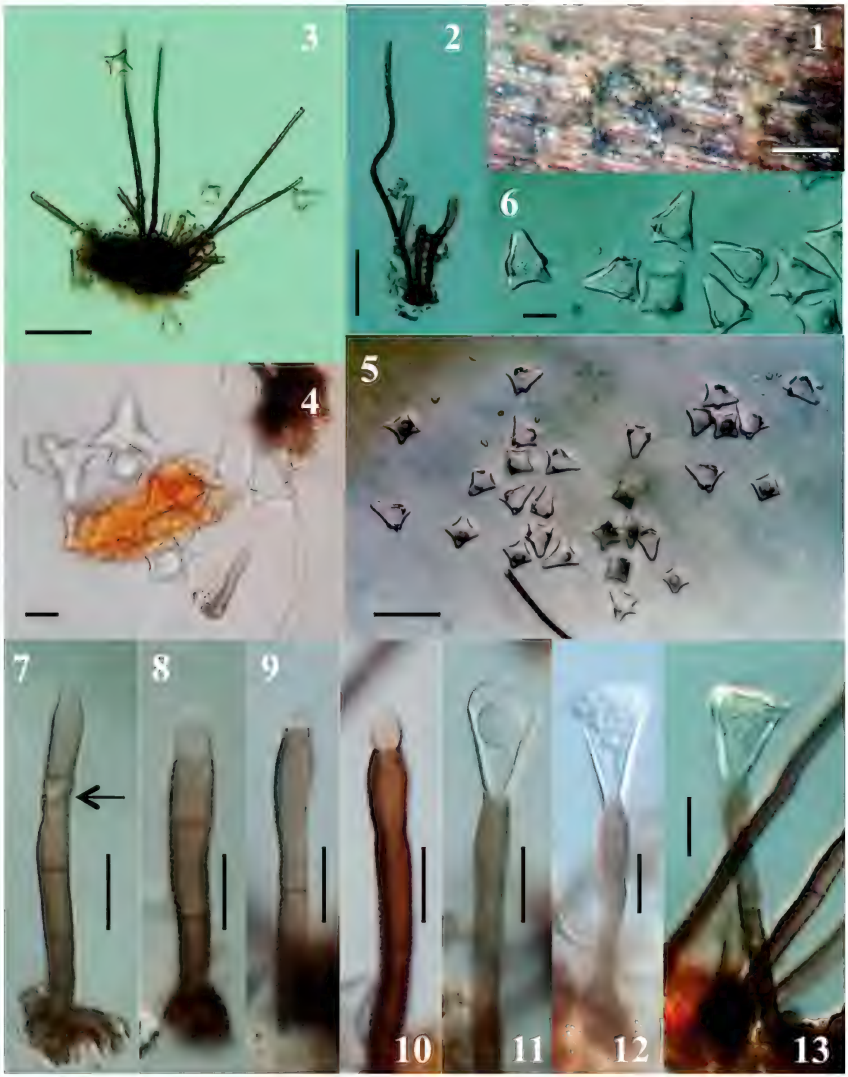
Differs from other species of *Nawawia* in having conidia with 4(–5) distal setulae but no basal setula.

Type: Malaysia. Perak, Menglembu, Bukit Kledang, on submerged wood, 10 November 2013, Wai-Yip Lau (**Holotype**, UTAR(M)-0004).

Etymology: *quadrisetulata*, refers to the conidium that usually bears four setulae.

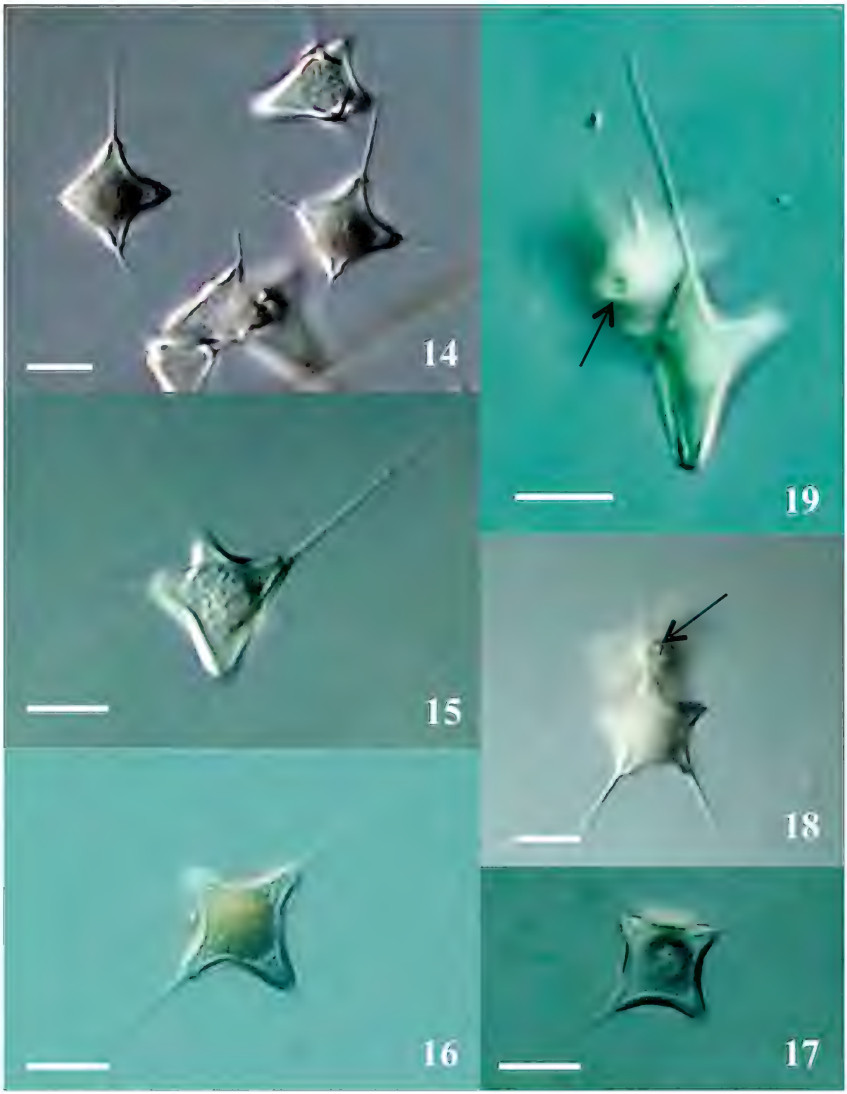
Colonies on the natural substratum effuse, hairy, dark brown, with glistening mass of conidia at the tip of conidiophores. Mycelium partly immersed and partly superficial, composed of pale brown, septate hyphae 2–3 µm wide. Stroma absent or meager (≤40 µm wide). Conidiophores cylindrical to slightly clavate, single or in small groups, erect, straight, unbranched, thick-walled, smooth, medium to dark brown, becoming paler towards the apex, often proliferating percurrently, 3–7 septate, 50–280 µm long, 5–8.5 µm wide. Conidiogenous cells integrated, terminal, phialidic, medium brown, smooth, cylindric-clavate 17–50 µm long, 6.5–10 µm wide at the widest part; collarettes proliferating without progression. Conidia hyaline, aseptate, smooth, thin-walled, 4(–5)-setulate, guttulate, obpyramidal, viewed from side turbinate-triangular, with 4(-5) blunt protruding edges at the broader distal end, viewed from above 4-lobed or cruciform with blunt protruding corners (bearing four setulae, one at each corner) and sometimes 5-lobed or stellate (bearing five setulae, one at each protruding corner), 30–37.5 µm long and 22.5–32.5 µm wide, base obconically truncate, with a depressed hilum 5–7.5 µm wide; setulae stiff, hyaline, 30–57.5 µm long, ca. 0.5 µm wide.

Comments— The conidia of this species bear 4–5 setulae at the distal end, and thus they are unique in shape. At least 100 conidia of this species were examined; the majority (93%) had 4 setulae and a few (7%) had 5 setulae. We have attempted unsuccessfully to grow this species in culture, using single-spore isolation method described by Goh (1999). Isolated conidia were inoculated to water agar, malt extract agar, cornmeal agar, and oatmeal agar, but none germinated.



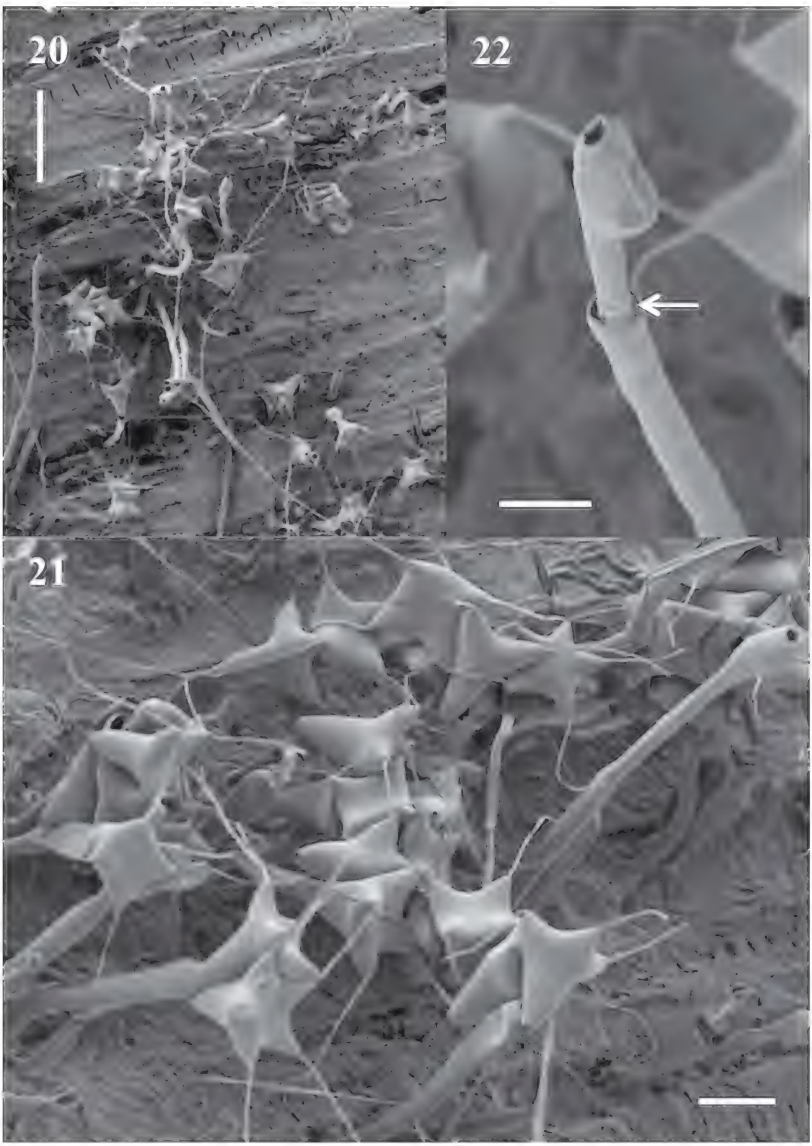
Figs 1–13. *Nawawia quadrisetulata* (holotype). 1. Colonies on natural substratum. 2, 3. Conidiophores. 4. Conidia under bright-field microscopy. 5, 6. Conidia under differential interference contrast (DIC) microscopy. 7. Conidiophore percurrent proliferation (arrowed). 8–13. Conidiogenous cell showing sequential development of conidia. Scale bars: 1 = 100 µm; 2, 3, 5 = 50 µm; 4, 6–13 = 20 µm.

A synopsis of the six species is presented in Table 1. Four of the previously described species (*Nawawia dendroidea* K.D. Hyde et al., *N. filiformis* (Nawawi)



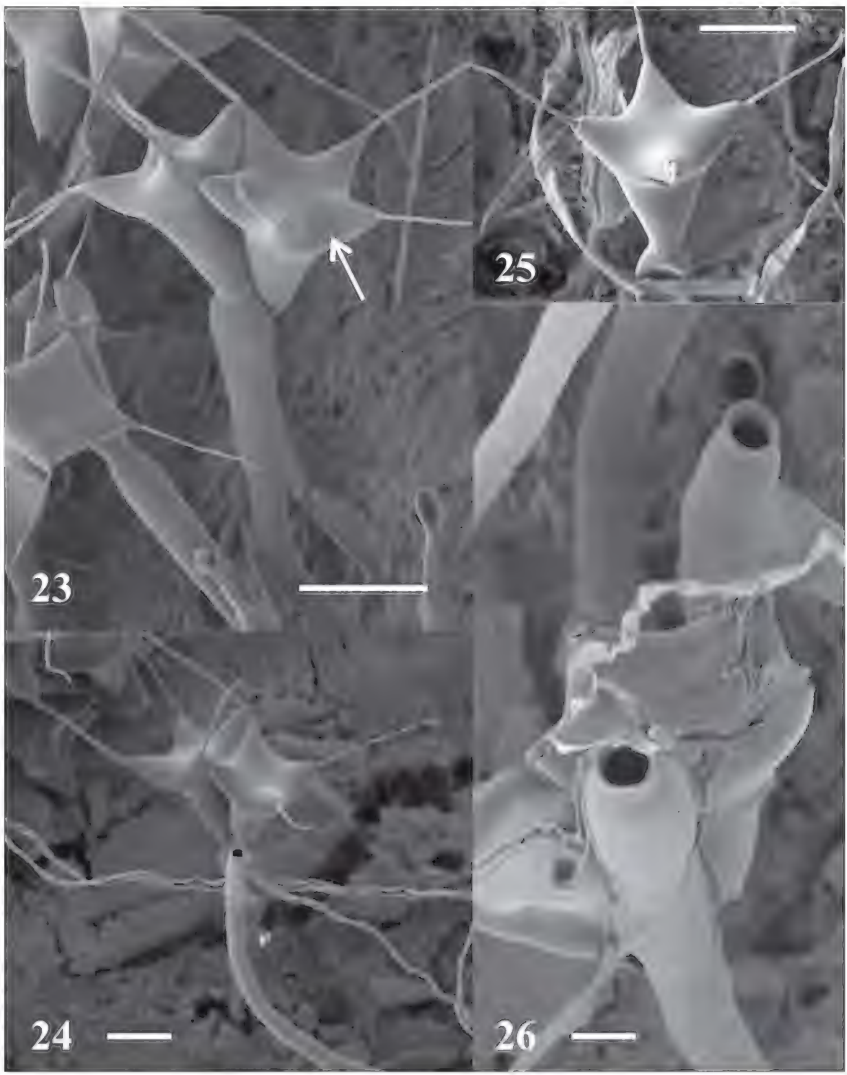
Figs 14–19. *Nawawia quadrisetulata* (holotype): conidia under DIC microscopy. 14. Quadrisetulate conidia. 15. Side-view of a conidium. 16. A conidium with a yellowish mass of oil in the cell. 17. A quadrisetulate conidium. 18. A conidium with 5 setulae; arrow points to the basal hilum of another conidium in the vicinity. 19. Conidia, with one conidium showing the basal hilum (arrowed). Scale bars = 20 µm.

Marvanová, *N. nitida* Kuthub. et al., and *N. sasae-kurilensis* Melnik & K.D. Hyde) have tetrahedral to obpyramidal conidia with 3 distal setulae (Hyde et



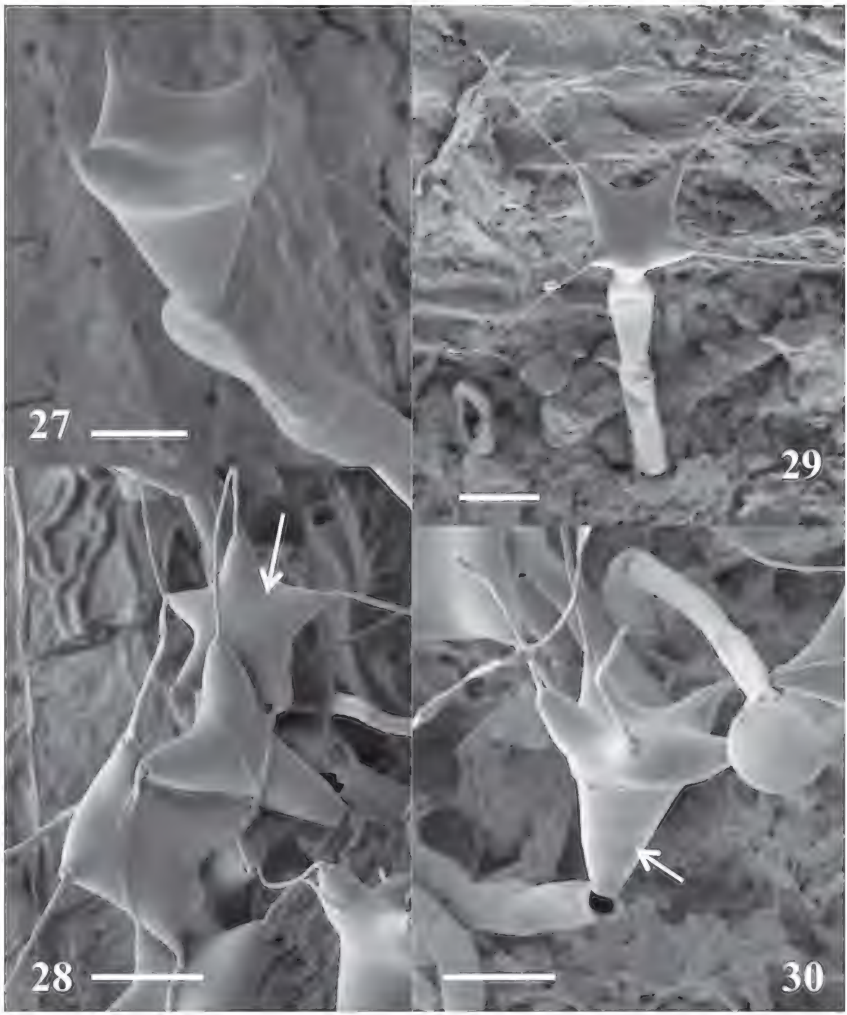
Figs 20–22. *Nawawia quadrisetulata* (holotype): SEM. 20. Colonies on surface of the natural substratum. 21. Conidiophores and conidia on natural substratum. 22. Phialide and percurrent proliferation (arrowed). Scale bars: 20 = 50 µm; 21, 22 = 20 µm.

al. 1996; Kuthubutheen et al. 1992; Marvanová 1980; Mel'nik & Hyde 2006). Conidia of *N. malaysiana* Crous & S.S. Lee are 5-lobed and bear 4 distal setulae



Figs 23–26. *Nawawia quadrisetulata* (holotype): SEM. 23. Conidia produced from conidiophores. Arrow points to a conidium with 5 setulae. 24, 25. Quadrisetulate conidia produced at the apex of conidiophores. 26. Conidiophores showing the terminal phialides. Scale bars: 23–25 = 20 μm; 26 = 5 μm.

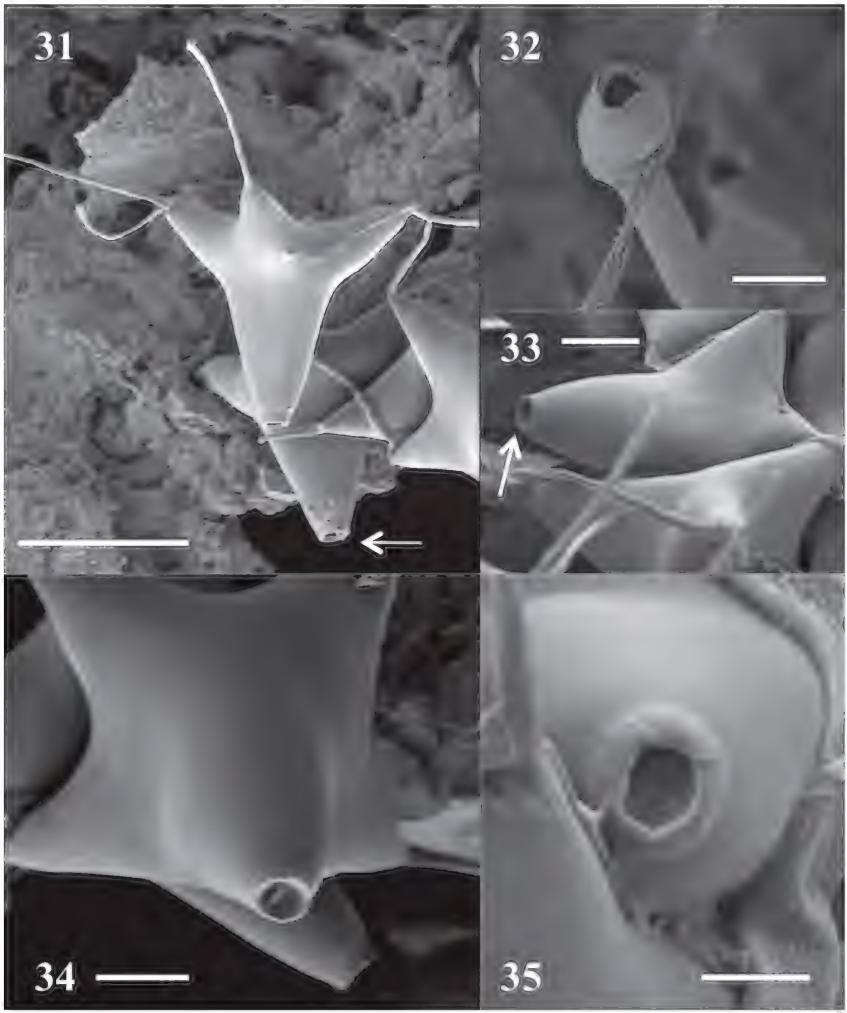
plus a basal setula (Crous et al. 2009), whereas *N. quadrisetulata* conidia lack a basal setula. Moreover, *N. malaysiana* differs from the other five species in being sporodochial, producing conidia that are distinctly smaller and fusoid to ellipsoid in side-view, and it was isolated from the apex of a conidiomatal



Figs 27–30. *Nawawia quadrisetulata* (holotype): SEM. 27. Conidium with the corners yet to form the setulae. 28. Conidia; arrow points to one that have 5 setulae. 29. Conidium attached to the apex of conidiophore. 30. A 5-setulate conidium (arrowed) at the opening of phialide. Scale bars = 5 μm.

spore mass of a foliicolous *Satchmopsis* species. Compared with the other five *Nawawia* species, *N. malaysiana* is atypical and its taxonomic placement in the genus is doubtful.

Under the scanning electron microscope (SEM), the conidial hilum of *N. quadrisetulata* is depressed and has a distinct rim, suggesting that the



Figs 31–35. *Nawawia quadrisetulata* (holotype): SEM. 31. Conidia; arrow points to position of hilum. 32. Phialide of conidiophore. 33, 34. Conidia showing the basal hilum (arrowed). 35. Conidial hilum. Scale bars: 31 = 20 μm; 32–34 = 10 μm; 35 = 5 μm.

conidial secession is rhexolytic, whereas the conidial hilum in *N. dendroidea* appeared to be flush with the obconically truncate base (Hyde et al. 1996), suggesting a schizolytic conidial secession. In the other four *Nawawia* spp., hilum morphology was neither reported in detail nor studied under the SEM.

Among the six *Nawawia* species, only *N. nitida* has conidiogenous cells that are doliiform and with distinctly flared collarettes similar to those found in

Table I. Synopsis of *Nawawia* species

Species	Conidiophores	Conidia	In vitro culture	Substratum	Original locality	Reference
N. dendroidea	Synnematous; 350–800 × 3–6 µm	Round-tetrahedral or obpyramidal; mostly with 3 apical setulae (4–8 µm long); 10–12 × 8–10 µm	Attempts unsuccessful	Submerged wood	Australia	Hyde et al. 1996
N. filiformis	Solitary; 70–315 × 4–8 µm	Turbinate to triangular; mostly with 3 apical setulae (15–34 µm long); 13–18 × 14–18 µm; with a *Chloridium* synanamorph in culture	Growth on MEA*	Submerged petioles and twigs	Malaysia	Nawawi 1973, Marvanová 1980
N. malaysiana	Sporodochial; 10–40 × 3–4 µm	5-lobed; with 4 apical & 1 basal setulae (3–9 µm long); 4–6 × 4–6 µm	Growth on MEA and OMA*	Associated with *Satchmopsis* sp, on *Eucalyptus* leaf	Malaysia	Crous et al. 2009
N. nitida	Solitary, short, bearing doliiform phialides; 25–75 × 10–12 µm	Round-tetrahedral or obpyramidal; with 3 apical setulae (38–74 µm long); 29–35 × 20–26 µm	Attempts unsuccessful	Submerged twigs	Malaysia	Kuthubutheen et al. 1992
N. quadrisetulata	Solitary or fasciculate; 50–280 × 5.5–10 µm	4–5-lobed; mostly with 4 apical setulae (30–57.5 µm long); 30–37.5 × 22.5–32.5 µm	Attempts unsuccessful	Submerged wood	Malaysia	This paper
N. sasae-kurilensis	Solitary, 160–240 × 5–10 µm	Round-tetrahedral; with 3 apical setulae (4–6 µm long); 10–14 × 8–11.5 µm	Not reported	Dead culm of *Sasa kurilensis* (Russian bamboo)	Russia	Mel'nik & Hyde 2006

* MEA = malt extract agar; OMA = oatmeal agar

species of *Obeliospora* Nawawi & Kuthub. (Nawawi & Kuthubutheen 1990). A *Chloridium* synanamorph (producing ellipsoid, non-setulate spores) was reported in cultures of *N. filiformis* (Nawawi 1973, Wu & McKenzie 2003), but synanamorphs are not known in the other five *Nawawia* species.

Acknowledgements

The authors are grateful to the Universiti Tunku Abdul Rahman (UTAR) for financial support (IPSR/RMC/UTARRF/2012-C2/G04) to study the diversity of fungi in Malaysia. We would like to thank the two peer reviewers of this paper, Dr. R.F. Castañeda Ruiz (Instituto de Investigaciones Fundamentales en Agricultura Tropical (INIFAT), Cuba) and Dr. Clement K.M. Tsui (Department of Forest Sciences, University of British Columbia, Canada) for their valuable comments on the manuscript. Mr. Ooh Keng Fei (Faculty of Science, UTAR—Perak Campus) is thanked for his technical assistance in the scanning electron microscopy. Thanks are extended to Miss Loh Bao Yee in the faculty for her technical assistance in photographing the fungi.

Literature cited

Crous PW, Groenewald JZ, Lee SS. 2009. *Nawawia malaysiana*. Fungal Planet 41, Persoonia 23: 194-195.

Goh TK. 1999. Single-spore isolation using a hand-made glass needle. Fungal Diversity 2: 47–63.

Hyde KD, Goh TK, Steinke T. 1996. *Nawawia dendroidea*, a new synnematous hyphomycete from submerged *Phragmites* in South Africa. Mycological Research 100: 810–814. http://dx.doi.org/10.1016/S0953-7562(96)80026-8

Index Fungorum. 2014. http://www.indexfungorum.org/names/Names.asp (Accessed 23 Sep. 2014).

Kuthubutheen AJ, Liew GM, Nawawi A. 1992. *Nawawia nitida* sp. nov. (hyphomycetes) and further records of *Nawawia filiformis* from Malaysia. Canadian Journal of Botany 70: 96–100. http://dx.doi.org/10.1139/b92-013

Marvanová L. 1980. New or noteworthy aquatic hyphomycetes: *Clavatospora*, *Heliscella*, *Nawawia* and *Heliscina*. Transactions of the British Mycological Society 75: 221–231. http://dx.doi.org/10.1016/S0007-1536(80)80083-0

Mel'nik VA, Hyde KD. 2006. *Nawawia sasae-kurilensis* sp. nov. from the Russian Far East. Mikologiya i Fitopatologiya 40: 411–414.

Nawawi A. 1973. *Clavatospora filiformis* sp. nov., an aquatic hyphomycete from Malaysia. Transactions of the British Mycological Society 61: 390–393. http://dx.doi.org/10.1016/S0007-1536(73)80163-9

Nawawi A, Kuthubutheen AJ. 1990. *Obeliospora*, a new genus of setose, phialosporus hyphomycetes with appendaged conidia. Mycotaxon 37:395–400.

Wu WP, McKenzie EHC. 2003. *Obeliospora minima* sp. nov. and four other hyphomycetes with conidia bearing appendages. Fungal Diversity 12: 223–234.

ISSN (print) 0093-4666 © 2014. Mycotaxon, Ltd. ISSN (online) 2154-8889

MYCOTAXON

http://dx.doi.org/10.5248/129.119

Volume 129(1), pp. 119–148 *July–September 2014*

The *Entolomataceae* of the Pakaraima Mountains of Guyana 6: ten new species and a new combination in *Nolanea*

Terry W. Henkel*[1], M. Catherine Aime[2], David L. Largent[1], & Timothy J. Baroni[3]

[1]*Department of Biological Sciences, Humboldt State University, Arcata, California, USA. 95521*
[2]*Department of Botany & Plant Pathology, Purdue University, West Lafayette, Indiana, USA. 47907*
[3]*Department of Biological Sciences, State University of New York—College at Cortland, New York, USA. 13045*
*Correspondence to: *twh5@humboldt.edu*

Abstract — Eleven *Nolanea* species are described from Guyanese collections. The West African *N. acuta* comb. nov. is reported for the first time from the Neotropics, and *N. alboproxima*, *N. applanata*, *N. clavata*, *N. claviformis*, *N. concentrica*, *N. furcata*, *N. mimiae*, *N. rava*, *N. sinuolata*, and *N. subsulcata* are new to science. All of these species occur in tropical rainforests, either in Upper Potaro River Basin in Guyana's Pakaraima Mountains or in the interior lowlands, and are presumed forest litter saprotrophs. Macromorphological, micromorphological, and habitat data and comparisons with similar taxa are provided for each. The genus *Nolanea* has not been previously reported from Guyana.

Key words — *Agaricales, Agaricomycetes,* Guayana Highlands, Guiana Shield, saprotrophic fungi

Introduction

Species in the cosmopolitan genus *Nolanea* (Fr.) P. Kumm. are easily recognized as members of the *Entolomataceae* (*Agaricales*) due to their pink-pigmented basidiospores that are angular in all views. Fries (1821) erected *Agaricus* "tribus" *Nolanea* for pink-spored agarics with a mycenoid stature, and his concept, with refinements based primarily in micromorphological characters, has been accepted by many later agaricologists at either generic (e.g. Largent 1994; Largent & Baroni 1988; Dennis 1953, 1970; Pegler 1983, 1997) or subgeneric (Noordeloos 1980, 1992, 2004) rank.

Here we apply the generic concept of *Nolanea* sensu Largent (1994) diagnosed by mycenoid or collybioid (rarely tricholomatoid) basidioma stature;

typically glabrous (rarely innately appressed fibrillose) pileus; the pileipellis typically a cutis with 1–4 uninflated hyphal layers overlying the more inflated hyphae of the outer pileal trama; long-celled hyphal elements typically present in the pileal, lamellar, and stipe tramas; lipoid globules, brilliant granules, and pseudocystidia absent; clamp connections typically absent (occasionally rare or very scarce in the pileipellis); and a +5 urea concentration. Morphologically similar *Inocephalus* species can be most readily distinguished from *Nolanea* by a pileipellis that intergrades imperceptibly into the pileal trama, a 0 or 0.5 urea concentration, and (for most *Inocephalus* species) the combination of abundant lipoid bodies in the tramal hyphae, pseudocystidia in the hymenium, clamp connections in the pileipellis, and a distinctly fibrillose or squamulose pileus (Largent 1994).

The few molecular systematics studies published on *Entolomataceae* have proved rather equivocal regarding the status of *Nolanea* at the generic or subgeneric levels (e.g., Co-David et al. 2009; Baroni & Matheny 2011; He et al. 2013). Other preliminary molecular data support entolomatoid fungi with *Nolanea's* morphological diagnostic characters as a genus (Bergemann & Largent unpubl.). Given the current lack of clear, molecular-based resolution of genera within the *Entolomataceae*, we have used morphology to distinguish and describe the new taxa presented here.

New World tropical and subtropical entolomatoid species meeting the diagnostic requirements of *Nolanea* sensu Largent (1994) have been found in the Lesser and Greater Antilles (Murrill 1911; Pegler 1983), Trinidad and Venezuela (Dennis 1953, 1970), Brazil (Pegler 1997), and elsewhere in South America (Horak 1977).

We report here eleven *Nolanea* species for the first time from Guyana. One, *Nolanea acuta* comb. nov., was previously known from Gabon in West Africa; the other ten species are described as new to science: *N. alboproxima*, *N. applanata*, *N. clavata*, *N. claviformis, N. concentrica*, *N. furcata*, *N. mimiae*, *N. rava*, *N. sinuolata*, and *N. subsulcata*.

Materials & methods

Specimens were collected from the Upper Potaro River Basin during the May–August rainy seasons of 2000, 2003, 2005, 2007, 2009, 2010, and 2011 and the December rainy seasons of 2004 and 2009. The collecting area is located within a 15 km radius of a permanent base camp at 5°18′04.8″N 59°54′40.4″W, elevation 710–750 m, in an undulating valley approximately 15 km east of Mt. Ayanganna (2200 m) and is densely forested with a mosaic of primary *Dicymbe*-dominated and mixed forests of the *Eschweilera–Licania* association (Henkel 2003). Additional collections were made during the May–June 2011 rainy season from Guyana's Upper Demerara River Basin at Mabura Ecological Reserve within 2 km of a field station located at 5°09′19.0″N 58°41′58.9″W, elevation 100 m, in monodominant stands of *Dicymbe altsonii*.

Methods for field descriptions, microscopic analyses, and image capture follow Largent et al. (2008). The formula for Amman's solution is cited in Largent et al. (1977). Fungi were field-dried with silica gel. Color designations follow Kornerup & Wanscher (1978) with color plates noted in parentheses (e.g., 4A7). Specimens were deposited in the following herbaria: BRG, HSU, and PUL (Holmgren et al. 1990). Microscopic structures were measured as previously described (Largent 1994; Largent et al. 2008). Statistics determined include means of basidiospore length and width ± standard deviations; E = quotient of length by width indicated as a range variation in n objects measured; Q = the mean of E-values; n = number of objects measured.

Taxonomy

Nolanea acuta (Romagn. & Gilles) Largent, **comb. nov.** PLATE 1

MYCOBANK MB 519978

≡ *Rhodophyllus acutus* Romagn. & Gilles, Beih. zur Nova Hedwigia 59: 542 (1979)

PILEUS 21–32 mm broad, broadly convex with acute umbo; umbo and part of surrounding disc dark yellowish brown (5F8) because of appressed radial fibrils, background color lighter, maturing overall to light blond (4C3), non-hygrophanous; margin slightly inturned, somewhat rimose-frayed, not striate or translucent-striate. LAMELLAE subclose, subthick, very finely adnexed, becoming pinkish from spores (6B4); lamellulae 3 between lamellae, in 2 tiers. STIPE 70–85 × 3 mm wide at apex, 6 mm at base, tapered upward, whitish with appressed brown longitudinal fibrils. BASAL MYCELIUM scant, white, appressed felty. STIPE CONTEXT white, stuffed. ODOR and TASTE none.

BASIDIOSPORES distinctly 5–7-angled in profile, dorsal and ventral views, heterodiametric in all views, 7.8–11.3 × 5.2–7.5 µm (mean = 9.7 ± 0.83 × 6.7 ± 0.59 µm; E = 1.3–1.8, Q = 1.43 ± 0.09; n = 30); hilar appendage distinct. BASIDIA clavate, slightly to strongly tapered at the base, 27.7–33.1 × 7–10 µm (mean = 30.2 ± 1.8 × 8.6 ± 0.88 µm; E = 3.0–4.4, Q = 3.53 ± 0.30; n = 11), 4-sterigmate. CHEILOCYSTIDIA AND PLEUROCYSTIDIA absent. LAMELLAR TRAMA of long-celled, parallel to subparallel hyphae (not measured). PILEIPELLIS a cutis of uninflated hyphae that are 3.2–10.7 µm wide and rarely uplifted; TERMINAL CELLS cylindric to cylindro-clavate, 33.6–73.8 µm long. PILEUS TRAMA HYPHAE inflated beneath the pileipellis, 5–20 µm wide, elsewhere 5–40 µm wide. STIPITIPELLIS a cutis; hymenial clusters and caulocystidia absent. PIGMENTATION uniform, light to medium brown in the cytoplasm of the pileipellis; incrusted hyphae absent. REFRACTIVE HYPHAE abundant in the pileus trama. CLAMP CONNECTIONS absent. MICROCHEMICAL REACTIONS in Amman's solution: hymenial elements strongly cyanophilic.

ECOLOGY, RANGE, DISTRIBUTION — Scattered on soil in *Dicymbe corymbosa* forest in the Upper Potaro River Basin of Guyana; originally described from Gabon.

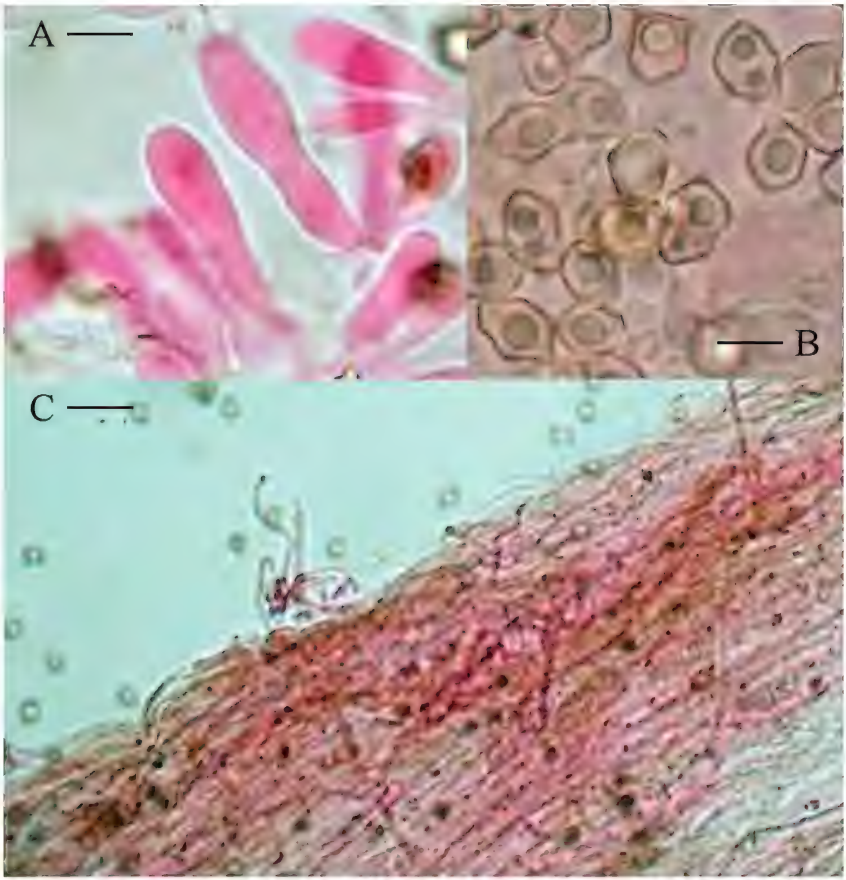


Plate 1. *Nolanea acuta* (Aime 1314). A. Basidia. B. Basidiospores. C. Pileipellis. Bars = 10 µm.

Specimen examined. **GUYANA. Region 8: Potaro-Siparuni.** Pakaraima Mountains. Upper Potaro River Basin, ~15 km east of Mt. Ayanganna, environs of base camp located on Potaro River one km upstream from confluence with Whitewater Creek at 5°18′04.8″N 59°54′40.4″W, elevation 710–750 m; vicinity of base camp, on soil, 21 June 2000, Aime 1314 (BRG; PUL).

Comments — *Nolanea acuta* is distinguished by its combination of relatively large basidiomata, a dark yellowish brown acutely umbonate pileus, clavate stipe with brownish longitudinal fibrils on a whitish ground, heterodiametric basidiospores averaging 9.7 × 6.7 µm (Q = 1.4), cytoplasmic pigmentation, and lack of hymenial cystidia. *Rhodophyllus acutus* from Gabon (Romagnesi & Gilles 1979) is morphologically identical to Guyanese *N. acuta*, justifying the new combination. The discovery of *N. acuta* in Guyana constitutes a significant intercontinental range extension for the species.

Nolanea acuta is similar to *N. solstitialis* (Fr.) P.D. Orton [≡ *Entoloma solstitiale* (Fr.) Noordel.] in pileus and stipe size, shape, and colors, basidiospore shape and size, and lack of cheilocystidia, but *N. solstitialis* can be distinguished by its translucent-striate hygrophanous pileus and smooth glabrous stipe (Noordeloos 1980, 1992, 2004).

Because of its basidioma colors, acutely umbonate pileus, and lack of cheilocystidia, *Nolanea acuta* resembles members of a species complex centered around *N. papillata* Bres., including *N. pseudopapillata* Pegler [= *N. papillata* sensu Dennis. non Bresadola], *N. mammifer* (Romagn.) Pegler [≡ *Rhodophyllus mammifer* Romagn.], and *N. apiculata* Petch [≡ *Entoloma acuminatum* E. Horak]. All these species differ from *N. acuta* primarily by their externally encrusting pileipellis pigments (Dennis 1970; Horak 1980; Pegler 1983).

Nolanea alboproxima Largent, Aime & T.W. Henkel, **sp. nov.** PLATE 2

MYCOBANK MB 519972

Differs from *Nolanea proxima* by its whitish overall coloration, heterodiametric basidiospores and lack of incrusting pigment in the pileus tramal hyphae.

TYPE: Guyana. Region 8: Potaro-Siparuni. Pakaraima Mountains. Upper Potaro River Basin, ~20 km east of Mt. Ayanganna, Tadang Base Camp 2 km south of Potaro River at 5°16′14.5″N 59°50′39.1″W, elevation 710–750 m; vicinity of base camp, 21 December 2009, Henkel 9116 (BRG, **holotype**; HSU, **isotype**).

ETYMOLOGY: *albus* (L. adj.) = white; referring to the whitish pileus color that differentiates *N. alboproxima* from *N. proxima*.

PILEUS 6–16.5 mm broad, 3–4.5 mm high, broadly convex to plano-convex to plane, somewhat flattened across apex and subumbilicate, glabrous, translucent striate from disc to margin, overall pale orangish white (4A2–4A3) to greyish orange (4B4–4B5) with age, moist; disc hygrophanous with medium grey cast; margin irregularly crenulate. LAMELLAE subclose, subthin, adnexed to barely adnate, 3–5 mm long, 0.5–2.0 mm tall, off-white at first (4A1–4A2), developing slight pinkish overtones (~5A3) with age; margins concolorous, nearly smooth under hand lens; lamellulae consistently 3 between lamellae, 0.5–2 mm long, in 2 tiers. STIPE 25–53 × 1–2 mm at apex, 2–4 mm at base, tapering upward, translucent, off-white (4A1–4A2) to pale light yellow (4A4), slightly yellowing (3A3–3A4) where handled, glabrous, hollow. BASAL MYCELIUM scant, white, and hispid at extreme base. ODOR none; TASTE not noted.

BASIDIOSPORES distinctly 5–6(–7)-angled and heterodiametric in profile view, 7.7–10.8 × 5.3–7.8 µm (mean = 9.3 ± 0.7 × 6.8 ± 0.57 µm; E = 1.2–1.7, Q = 1.38; n = 58). BASIDIA clavate and tapering to a long, slender base, 30.0–42.8 × 7.1–13.1 µm (mean = 35.0 ± 4.0 × 9.3 ± 1.5 µm; E = 2.3–5.2, Q = 3.9 ± 0.86; n = 24), 2–4-sterigmate, abundant on the lamella edge. CHEILOCYSTIDIA and PLEUROCYSTIDIA absent. LAMELLAR TRAMA subparallel, relatively narrow; composed of long-celled hyphae, these 39.5–186.1 × 11.1–16.8 µm, poorly

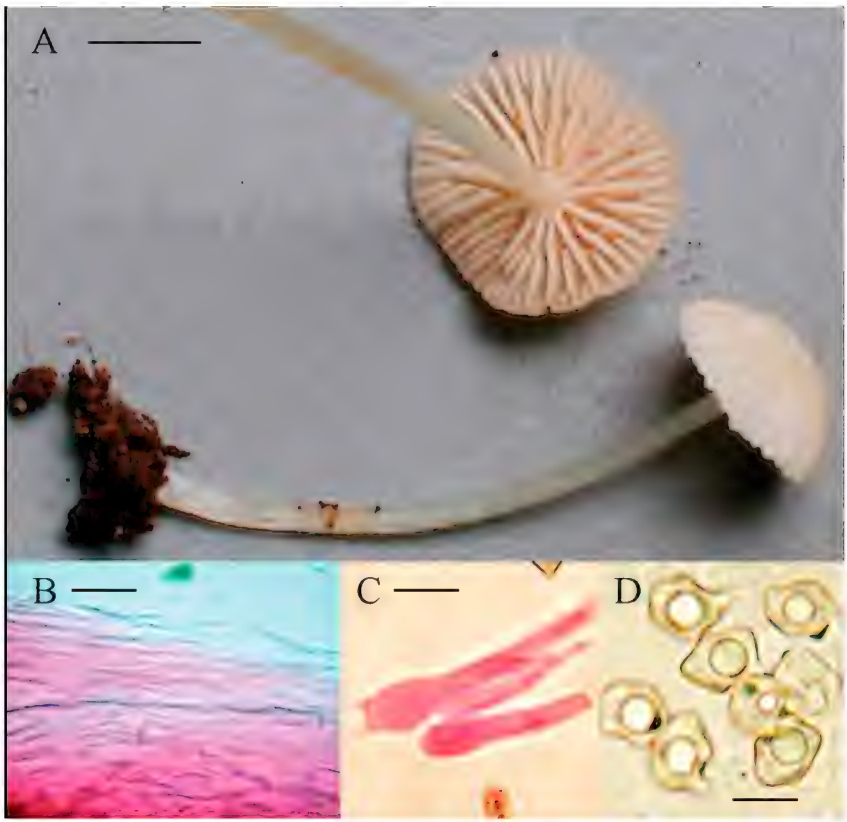


Plate 2. *Nolanea alboproxima* (holotype; Henkel 9116). A. Basidiomata. B. Pileipellis. C. Basidia. D. Basidiospores. Bars: A = 10 mm; B–D = 10 µm.

separating. Pileipellis a shallow cutis of 1–3 uninflated hyphal layers; hyphae 4–6 µm wide; terminal cells cylindric to cylindro-clavate, 45.9–180.9 × 4.2–6.7 µm. Pileal trama hyphae inflated beneath the pileipellis, 10–14 µm wide, elsewhere 96.8–273.4 × 8.6–24.8 µm. Stipitipellis a cutis; caulocystidia absent. Refractive hyphae abundant in the stipe trama, rare in the pileus trama, absent in the lamellar trama. Pigmentation minimal, apparently cytoplasmic, and not incrusting in the pileipellis. Clamp connections absent.

Ecology, range, distribution — Scattered on humus or seed husks in *Dicymbe* spp. forests; known only from the Upper Potaro River Basin of Guyana during the December–January rainy season.

Additional specimen examined. **GUYANA. Region 8: Potaro-Siparuni.** Pakaraima Mountains. Upper Potaro River Basin, ~15 km east of Mt. Ayanganna, environs of base camp located on Potaro River one km upstream from confluence with

Whitewater Creek at 5°18′04.8″N 59°54′40.4″W, elevation 710–750 m; on buried seed husk, 2 January 2004, Aime 2416 (BRG; PUL).

Comments — *Nolanea alboproxima* is distinguished by its small, plano-convex, pale yellowish white, translucent-striate pileus, pale yellow stipe, heterodiametric spores averaging 9.3 × 6.8 µm, and its lack of hymenial cystidia. Even though the basidioma colors are reminiscent of those predominant in *Alboleptonia*, *N. alboproxima* fits well in *Nolanea* because it combines a thin pileal cutis, inflated cells in the outermost pileal trama, and lack of clamp connections. Its overall aspect resembles an off-white form of *N. proxima* Largent or *N. cetrata* (Fr.) P. Kumm. Isodiametric basidiospores and externally encrusted pileus tramal hyphae distinguish *N. proxima* from *N. alboproxima*. *Nolanea cetrata* has similarly shaped and sized basidiospores but can be separated from *N. alboproxima* by its yellowish brown pileus, silvery striate stipe, bisterigmate basidia, and distinctive cytoplasmic or vacuolar pigment (Largent 1994).

Several other species representing other entolomatoid genera share similar basidioma color, basidiospore size, lack of odor, and lack of clamp connections with *Nolanea alboproxima*. Of these, *Entoloma purum* E. Horak & Desjardin from Hawaii is distinguished by its suede-like to pubescent pileus, trichodermial pileipellis, and much smaller basidiomata (pileus 4–10 mm broad, stipe 3–5 × 0.5–0.7 mm; Horak & Desjardin 1993). *Entoloma proprium* E. Horak from New Caledonia can be separated by its fimbriate lamellae, distinctive cheilocystidia, incrusting pigments, and abundant refractive hyphae (Horak 1980).

Rhodophyllus vetulus Romagn., *R. tortilis* Romagn., and *R. subsericellus* Pat. [≡ *R. platypus* Romagn, nom. illegit] from Madagascar and *R. flexuosipes* Romagn. & Gilles from Gabon resemble *N. alboproxima*. *Rhodophyllus vetulus*, although with a small pileus, resembles an *Entoloma* s. str. as it lacks inflated hyphae in the outer pileus trama and the stipe is >4 mm broad at the apex. *Rhodophyllus tortilis* is distinctive because of its leathery, somewhat scabrous pileus lacking striations, and overall lack of yellow tones in the basidiomata. *Rhodophyllus subsericellus* has a finely pilose, opaque pileus without striations (Romagnesi 1941, as *R. platypus*). *Rhodophyllus flexuosipes* is distinguished by its ≤70 mm long flexuous stipe and ≤35 mm broad campanulate pileus (Romagnesi & Gilles 1979).

Nolanea applanata Largent & T.W. Henkel, **sp. nov.** Plate 3

Mycobank MB 519974

Differs from *Entoloma bakeri* by its brownish orange, translucent-striate pileus and hollow stipe.

Type: Guyana. Region 8: Potaro-Siparuni. Pakaraima Mountains. Upper Potaro River Basin, ~15 km east of Mt. Ayanganna, environs of base camp located on Potaro River

one km upstream from confluence with Whitewater Creek at 5°18′04.8″N 59°54′40.4″W, elevation 710–750 m; 17 August 2007, Henkel 8872 (BRG, **holotype**; HSU, **isotype**).

Etymology: *applanatus* (L. adj.) = flat, referring to the pileus shape.

Pileus 45–55 mm broad, planate to upturned, with a low umbo, brownish orange (5C5) throughout, hygrophanous, becoming lighter first over disc and then outwards along radial striations, translucent-striate throughout except over the disc; surface appearing glabrous and moist throughout, under hand lens with extremely fine appressed radial fibrils; margin crenulate, irregularly splitting 1 or 2 times. Lamellae subdistant, subthick, abruptly adnexed, 20.5–24 mm long, 4–4.8 mm tall, pinkish tan (4A4–5A4); margins concolorous, finely and irregularly eroded under hand lens; lamellulae 1–2 between lamellae, 1–4 mm long. Stipe 62–97 × 4–7 mm (centrally), equal over apical ½, enlarging evenly over lower ½ to 9–11 mm wide at base, concolorous with pileus, glabrous to finely appressed fibrillose under hand lens over upper half, cartilaginous and twisting, with a hollow core. Basal mycelium scant, white, of matted hyphae. Odor none; taste minimal, slightly mealy.

Basidiospores distinctly 6–7(–8)-angled with the apex rounded and consistently heterodiametric in profile and dorsiventral views, 9.9–12.6 × 6.2–8.2 µm, (mean = 10.9 ± 0.6 µm × 7.1 ± 0.5 µm; E = 1.4–1.8, Q = 1.5 ± 0.1; n = 26). Basidia clavate, 36.3–47.3 × 6.5–12.2 µm, (mean = 43.8 ± 2.9 µm × 9.7 ± 1.3 µm, E = 3.5–4.9(–6.4), Q = 4.58 ± 0.7; n = 13), 2- or 4-sterigmate. Lamellar trama nearly parallel; hyphal cells very long, 149.4–542.8 × 9.0–18.4 µm; subhymenium narrow. Cheilocystidia and pleurocystidia absent. Pileipellis a cutis, 1–4 hyphal layers thick; hyphae 2–8 µm wide. Pileal trama hyphae inflated beneath the pileipellis, 8–18 µm wide, elsewhere very long-celled, 205–807 × 16.7–34.8 µm. Stipitipellis a cutis; hymenial clusters and caulocystidia absent. Refractive hyphae abundant immediately beneath the pileipellis, present but indistinct in the subhymenium, rare in the lamellar and stipe tramas. Pigmentation cytoplasmic, not incrusting in the pileipellis. Clamp connections absent.

Ecology, range, distribution — Occurring in a pair on root mat in *Dicymbe corymbosa* forest on sandy soils; known only from the type locality in the Upper Potaro River Basin of Guyana.

Comments — *Nolanea applanata* is distinguished by its dull flesh-tan to brownish orange, glabrous, hygrophanous, planate to upturned ≤55 mm broad pileus that is translucent-striate from disc to margin, concolorous stipe that is twisted, tapering upward, 60–97 × 4–7 mm (apex) to 9–11 mm (base), 6–8-angled basidiospores that average 10.9 × 7.1 µm, and absence of hymenial cystidia. Despite the rather large basidiomata, *N. applanata* fits well in *Nolanea* with its thin cuticular pileipellis, distinctly inflated outer pileal trama hyphae,

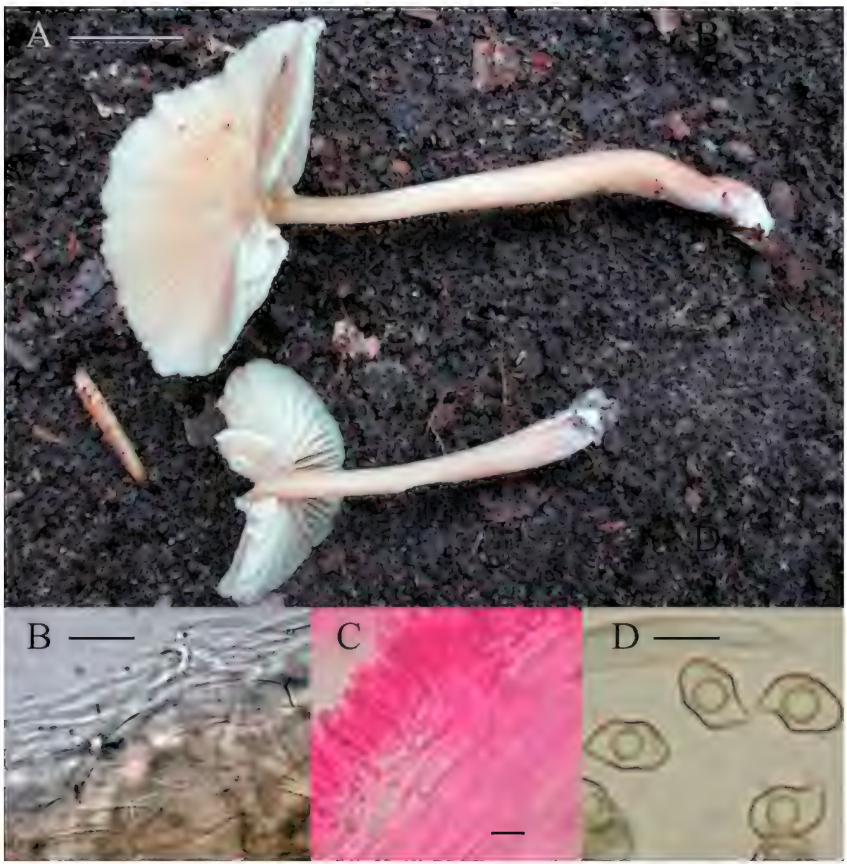


Plate 3. *Nolanea applanata* (holotype; Henkel 8872). A. Basidiomata. B. Pileipellis. C. Hymenium, basidia, and lamellar trama. D. Basidiospores. Bars: A = 10 mm; B–D = 10 µm.

very long-celled tramal hyphae in the pileus and stipe, as well as the translucent–striate pileus and twisted-striate, cartilaginous stipe.

While *Entoloma bakeri* Dennis from Martinique, Guadeloupe, and Trinidad is similar to *N. applanata* in basidioma size and stature, basidiospore dimensions, and absence of clamp connections, it can be distinguished by its cream–colored, silky-striate, innately fibrillose pileus and solid stipe (Pegler 1983).

Nolanea clavata Largent & T.W. Henkel, **sp. nov.** Plate 4

Mycobank MB 519975

Differs from *Entoloma conicoumbonatum* by its lack of incrusting pigments on the hyphae of the pileipellis and pileus trama and basidiospores with a higher length/width quotient.

Plate 4. *Nolanea clavata* (holotype; Henkel 8870). A. Basidiomata. B. Pileipellis. C. Basidia. D. Basidiospores. Bars: B = 10 mm; A, C, D = 10 µm.

Type: Guyana. Region 8: Potaro-Siparuni. Pakaraima Mountains. Upper Potaro River Basin, ~15 km east of Mt. Ayanganna, environs of base camp located on Potaro River one km upstream from confluence with Whitewater Creek at 5°18′04.8″N 59°54′40.4″W, elevation 710–750 m; vicinity of base camp, 17 August 2007, Henkel 8870 (BRG, **holotype**; HSU, **isotype**).

Etymology: *clavatus* (L. adj.) = club-shaped, referring to the stipe shape.

Pileus 26–28 mm broad, 16–21 mm high, narrowly to broadly conic with rounded to subumbonate apex, dull greyish brown to dark brown (6E4–6F4)

throughout, slightly darker brown over disc (6F5), appressed radially fibrillose throughout, fibrils more compact over disc but hardly erect, somewhat shiny macroscopically, opaque, not hygrophanous, silky under hand lens, dry to submoist; margin broadly undulating forming coarse irregular ridges between wide radial furrows; extreme edge crenulate and lighter greyish brown (~6C3). Lamellae subclose, subthick, deeply adnexed, 11.5–12.0 mm long, 4–5 mm tall, dull dirty pink; margins concolorous and smooth; lamellulae 1–2 between lamellae, 1–3 mm long. Stipe 33–50 mm × 3–4 mm (centrally), equal over apical ½, enlarging evenly over lower ½ to 6–8 mm, forming a subbulbous, rounded base, light grey brown (6C2–6D2) throughout but lighter over basal bulb, appearing silky sericeous, smooth but with fine longitudinal striations under hand lens. Basal mycelium a fine white bloom at extreme base. Odor none; taste strongly farinaceous.

Basidiospores distinctly 5–6-angled, apex rounded and heterodiametric in profile and dorsiventral views, 8.7–11.9 × 5.7–8.2 (mean = 10.2 ± 0.7 × 6.9 ± 0.5 µm; E = 1.4–1.7, Q = 1.5 ± 0.1; n = 30). Basidia (2–)4-sterigmate, short clavate, tapering basally, 31.1–41.0 × 8.5–12.3 µm (mean = 34.9 ± 2.9 µm × 10.6 ± 1.1 µm; E = 2.9–3.9, Q = 3.3 ± 0.2; n = 14). Cheilocystidia and pleurocystidia absent. Lamellar tramal hyphae subparallel and broad; cells 72.2–166.6 × 11.2–19.9 µm, E = 4.7–11.2. Pileipellis a cutis, 1–4 hyphal layers thick; hyphae 1.5–6 µm wide; upturned hyphal ends rare to absent. Pileus tramal hyphae slightly interwoven, inflated beneath the pileipellis, 15–20 µm wide, elsewhere 53.6–132.7 × 15.4–32.4 µm (E = 1.9–5.0). Stipitipellis a cutis; hymenial clusters and caulocystidia absent. Stipe trama hyphae long and broad, 104.8–412.2 × 13.5–24.6 µm, (E = 4.3–30.5). Pigmentation not observed in water mounts, not obvious in 3% KOH, likely cytoplasmic and uniform in the suprapellis of the pileus; not obvious in the stipitipellis. Refractive hyphae in radial section abundant in the pileal trama near the subhymenium; rare to absent elsewhere. Clamp connections absent.

Ecology, range, distribution — Solitary on humic layer of forest floor under *Dicymbe corymbosa*; known only from the type locality in the Upper Potaro River Basin of Guyana.

Comments — *Nolanea clavata* is distinguished by its relatively large basidioma with conical, subumbonate, grey brown to dark brown, radially appressed-fibrillose pileus, clavate, light grey brown stipe, strongly farinaceous taste, absence of hymenial cystidia, and heterodiametric, 5–6-angled basidiospores averaging 10.2 × 6.9 µm. The radially appressed–fibrillose, tall, conic pileus and clavate stipe of *N. clavata* are reminiscent of species of the entolomatoid genus *Inocephalus*. However, *N. clavata* lacks lipoid globules and clamp connections, features typical of *Inocephalus* species, and has a thin pileipellis of repent hyphae overlying distinctly inflated tramal hyphae, features diagnostic for *Nolanea*.

Because of the similar pileus shape and color, basidiospore size, and lack of cheilocystidia, *N. clavata* resembles *Entoloma substrictior* (Singer) E. Horak from Argentina and Chile and *Entoloma cystopus* (Berk.) Sacc. from India and Malaysia. However, *E. substrictior* has much larger basidiomata with a ≤50 mm broad strongly striate pileus with a rugose surface (Horak 1977). *Entoloma cystopus* is also much larger, with a pileus up to 45 mm broad and stipe up to 140 mm long, and becomes pale livid yellow when dry (Horak 1980). *Entoloma conicoumbonatum* Hesler resembles *N. clavata* in basidioma size and colors and has similarly shaped basidiospores, but differs in having incrusting pigments on the hyphae of the pileipellis and pileus trama and basidiospores with an E = 1.1–1.3 and Q = 1.15 (Hesler 1967; Noordeloos 1988).

Nolanea claviformis Largent & Aime, **sp. nov.** PLATE 5

MYCOBANK MB 805593

Differs from all described species of *Nolanea* by its light brown, concentrically ridged pileus, orange-white lamellae, dark brown stipe, clavate cheilocystidia, and two types of pileus pigment.

TYPE: Guyana. Region 10: Upper Demerara-Berbice. Mabura Ecological Reserve: ~100 m northwest of Mabura field station at 5°09′19.0″N 58°41′58.9″W, elevation 100 m; in *Dicymbe altsonii* monodominant stand #1, 23 May 2011, Aime 4279 (BRG, **holotype**; PUL, **isotype**).

ETYMOLOGY: *claviformis* (L. adj.) = club-shaped, referring to the clavate cheilocystidia.

PILEUS 7–25 mm diam, 7–17 mm high, campanulate-conical, concentrically ridged with a broad umbo, satiny light brown (5D4), felty, with appressed longitudinal silky fibrils under hand lens; margin suggestively striate, crimped, and felty. LAMELLAE 2 mm tall, narrow, adnate, close to crowded, orange white (5A2); margins broadly scalloped under hand lens; lamellulae numerous, difficult to count. STIPE 25–69 × 1.5–2.5 mm, equal, concolorous with pileus, silky; pruina scant at apex under hand lens. BASAL MYCELIUM appressed, white, extending upward. CONTEXT thin, hollow in stipe. BRUISING REACTIONS none. ODOR none; TASTE none, mealy in texture.

BASIDIOSPORES distinctly 4–5(–6)-angled in all views, isodiametric in polar view, isodiametric to heterodiametric in profile view, 7.3–10.9 × 6.8–8.6 µm (mean = 9.0 ± 0.8 × 7.5 ± 0.48 µm, E = 1.0–1.4, Q = 1.20 ± 0.10; n = 31). BASIDIA clavate to cylindro-clavate, 28.2–42.5 × 9.5–13.0 µm (mean = 35.2 ± 4.8 × 10.7 ± 1.2 µm, E = 2.9–3.9, Q = 3.28 ± 0.4; n = 7), 4-sterigmate. CHEILOCYSTIDIA abundant, forming a sterile layer, clavate, colorless, 41.4–57.6 × 8.7–15.7 µm (E = 4.0–4.8; n = 6). PLEUROCYSTIDIA absent. LAMELLAR TRAMA HYPHAE subparallel, cells relatively short and broad, 50.8–130.0 × 9–14.0 µm (n = 4). PILEIPELLIS a cutis, 4–5 hyphal layers thick; hyphae 2.0–7.5 µm wide; TERMINAL CELLS cylindric to cylindro–clavate, narrow. PILEAL TRAMA HYPHAE slightly inflated beneath the pileipellis; cells 65.9–128.2 × 8.7–14.6 µm,

Plate 5. *Nolanea claviformis* (holotype, Aime 4279). A. Basidiomata. B. Basidiospores. C. Cheilocystidia. D. Pileipellis. E. Caulocystidia. Bars: A = 10 mm; B–D = 10 µm.

elsewhere up to 20 µm wide. Stipitipellis a cutis between scattered hymenial clusters at the apex; terminal cells in hymenial clusters clavate, colorless,

similar to basidia but more narrow, 30.9–43.4 × 4.7–12.2 µm (E = 3.6–6.6, Q = 5.0; n = 5). Stipe trama hyphae long; cells 72.3–768.4 × 13.9–30.1 µm (n = 4). Refractive hyphae absent. Pigmentation minutely to distinctly incrusting the walls of the more slender hyphae in the pileal trama, faintly cytoplasmic in the pileipellis hyphae. Clamp connections absent.

Ecology, range, distribution — Scattered on litter of forest floor in *Dicymbe altsonii* forests on sandy soils; known only from the type locality at Mabura Ecological Reserve, Guyana.

Comments — *Nolanea claviformis* is characterized by its concentrically ridged pileus, narrow crowded lamellae, and two types of pigment in the pileipellis.

Refer to the comments sections for *N. sinuolata* and *N. concentrica* for discussions of species similar to *N. claviformis*.

Nolanea concentrica Largent & T.W. Henkel, **sp. nov.** Plate 6

Mycobank MB 519976

Differs from *Nolanea bicoloripes* by its abundant cheilocystidia and much shorter stipe.

Type: Guyana. Region 8: Potaro-Siparuni. Pakaraima Mountains. Upper Potaro River Basin, 15–20 km east of Mt. Ayanganna, Tadang Base Camp 2 km south of Potaro River at 5°16′14.5″N 59°50′39.1″W, elevation 710–750 m; vicinity of base camp, 19 December 2009, Henkel 9106 (BRG, **holotype**; HSU, **isotype**).

Etymology: *concentricus* (L. adj.) = ringed, referring to the concentrically ridged, umbonate pileus umbo.

Pileus 18 mm broad, 7 mm tall, broadly conic with concentrically ridged umbo, apex submammilate but rounded, brown (6D5–6D6), slightly darker (6E6) over disc, shiny sericeous and moist throughout, finely translucent-striate over outer 2/3; margin finely roughened. Lamellae subthin, crowded, adnate, 8.3 mm long, 1.5–2.0 mm tall, pink; margins concolorous, roughened-cystidiate under hand lens. Stipe 42 mm × 1.5 mm, equal, tan brown (5C4–5C5) throughout, smooth with fine suberect fibrils under hand lens. Basal mycelium lacking. Odor mildly fungoid; taste not obtained.

Basidiospores distinctly 4–5-angled in all views, isodiametric in polar view, in profile and dorsiventral views isodiametric to heterodiametric, 6.5–9.0 × 5.1–6.9 µm (mean = 7.7 ± 0.64 × 6.0 ± 0.52 µm; E = 1.13–1.61, Q = 1.3 ± 0.13; n = 28). Basidia cylindro-clavate, 24.2–30.6 × 6.7–9.5 µm (mean = 27.4 ± 2.0 × 8.1 ± 0.8 µm; E = 3.4 ± 0.47, Q = 2.8–4.2; n = 13), tapering abruptly near base to 1.5–4.6 µm wide, 4-sterigmate; sterigmata 1.9–4.3 µm long. Cheilocystidia abundant, forming a sterile layer, hyaline, broadly aciculate to obclavate at first, ultimately long ventricose-rostrate, 34.4–121.9 × 5.8–16.1 µm (mean = 79.3 × 10.3 µm; E = 7.75, Q = 5.9–9.6; n = 10). Pleurocystidia absent. Lamellar trama hyphae subparallel, cells relatively short and broad, 69.8–121.7 × 10.1–13.7 µm. Pileipellis a cutis, 4–5 hyphal layers thick; hyphae 2.0–6.2 µm

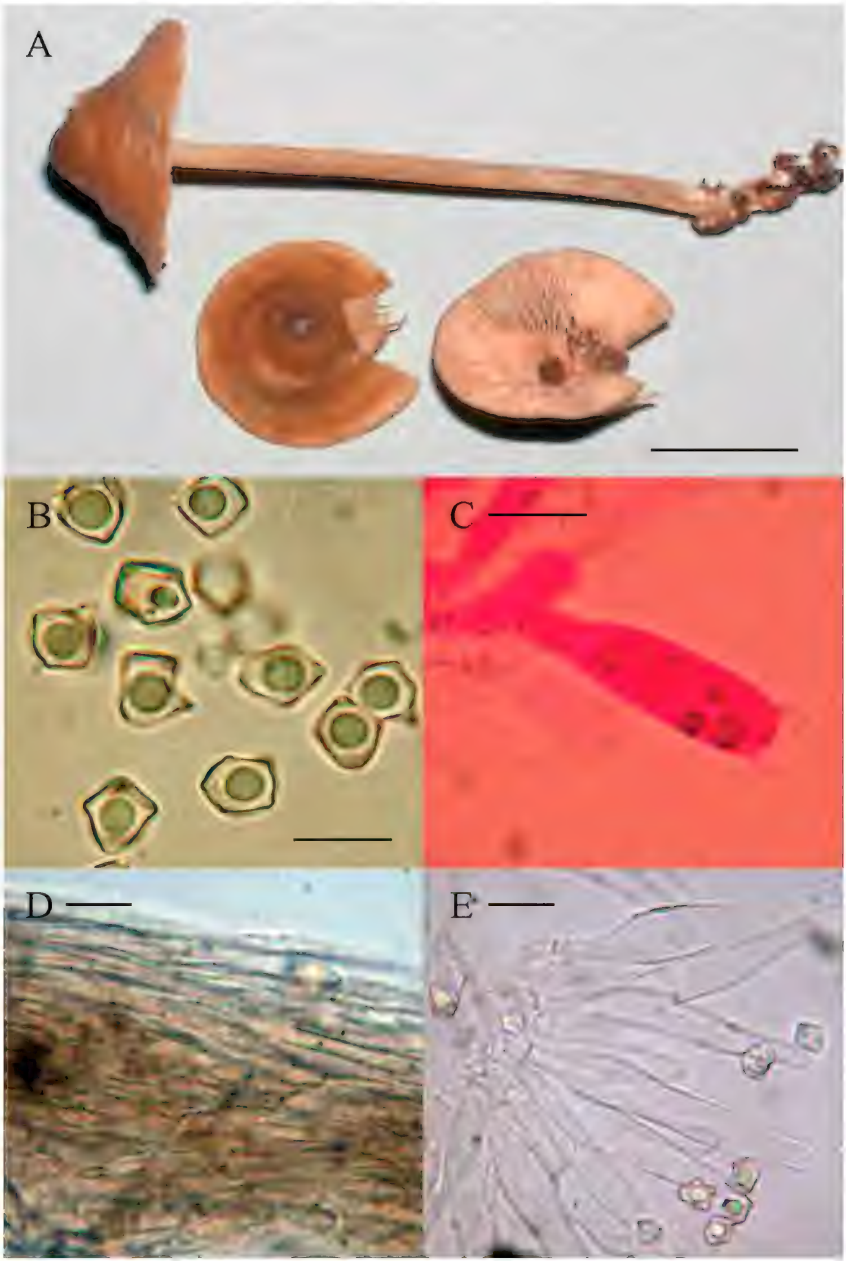


Plate 6. *Nolanea concentrica* (holotype; Henkel 9106). A. Basidioma and pilei. B. Basidiospores. C. Basidium. D. Pileipellis and pileal trama. E. Cheilocystidia. Bars: A = 10 mm; B–D = 10 µm.

wide; TERMINAL CELLS cylindric to cylindro-clavate. PILEAL TRAMA HYPHAE beneath the pileipellis slightly inflated, 8.7–14.6 µm wide; cells elsewhere 50.9–128.2 × 6.7–13.8 µm. STIPITIPELLIS not studied. REFRACTIVE HYPHAE absent. PIGMENTATION minutely incrusting the more slender hyphae in the pileal trama, faintly cytoplasmic within the pileipellis hyphae. CLAMP CONNECTIONS absent.

ECOLOGY, RANGE, DISTRIBUTION — Solitary on litter of forest floor in mixed *Dicymbe* spp. forest; known only from the type locality in the Upper Potaro River Basin of Guyana.

COMMENTS — *Nolanea concentrica* is distinguished by its dark brown, broadly conic pileus that is shiny sericeous, finely translucent-striate, and submammilate with a concentrically ridged umbo, crowded narrow lamellae, smooth equal stipe, abundant broadly aciculate to ventricose-rostrate cheilocystidia, and 4–5-angled basidiospores averaging 7.7 × 6.0 µm. *Nolanea bicoloripes* Largent & Thiers from western North America is similar to *N. concentrica* in the distinctive, concentrically ridged, umbonate, silky-sericeous pileus, narrow equal stipe, and some micromorphological features, but differs in its longer (≤140 mm) stipe and lack of cheilocystidia (Largent 1994).

Both *N. claviformis* and the sympatric *N. concentrica* have a concentrically ridged or beveled pileus, white lamellae, 4–5-angled basidiospores, and incrusting and cytoplasmic pigments in the pileipellis. *Nolanea claviformis* is distinguished by its clavate cheilocystidia, larger (~9.0 × 7.5 µm) basidiospores, and larger (28.3–42 5 × 9.5–12.0 µm) basidia. See the comments under *N. sinuolata* for additional information.

Nolanea subsolstitialis Largent from Washington, *N. infula* (Fr.) Gillet from the Netherlands and Sweden, and *N. solstitialis* from Europe also have small basidiospores, a beveled or concentrically ridged pileus, and a subpellis that is only slightly inflated and lack clamp connections. However, all three species lack cheilocystidia and have different stipe dimensions than *N. concentrica*. In addition, *N. solstitialis* has a distinct, abundant external incrusting pigment, *N. solstitialis* has intracellular pigment, and *N. infula* possesses both pigment types (Noordeloos 1980; Largent 1994).

Nolanea furcata Largent & T.W. Henkel, **sp. nov.** PLATE 7

MYCOBANK MB 805594

Differs from *Entoloma infula* var. *chlorinosum* by its non-papillate pileus, faint chlorine odor, and abundant cheilocystidia.

TYPE: Guyana. Region 8: Potaro-Siparuni. Pakaraima Mountains. Upper Potaro River Basin, 15–20 km east of Mt. Ayanganna, Tadang Base Camp 2 km south of Potaro River at 5°16′14.5″N 59°50′39.1″W, elevation 710–750 m; 0.5 km SW of Tadang Base Camp, 24 December 2009, Henkel 9139 (BRG, **holotype**; HSU, **isotype**).

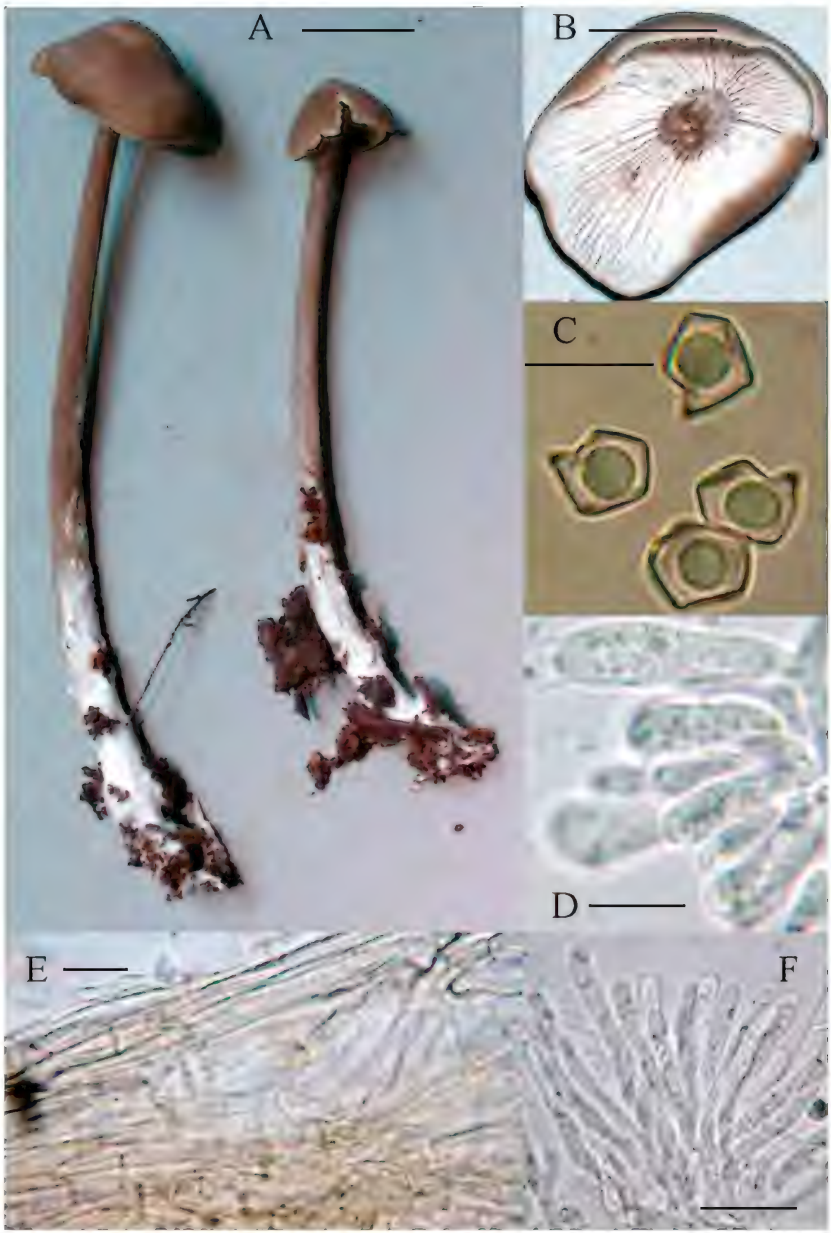


Plate 7. *Nolanea furcata* (holotype; Henkel 9139). A. Basidiomata. B. Hymenophore. C. Basidiospores. D. Basidia. E. Pileipellis and pileus tramal hyphae with external encrustations. F. Cheilocystidia. Bars: A, B = 10 mm; C–F = 10 µm.

Etymology: *furcatus* (L. adj.) = forked, referring to the irregularly forked lamellae.

Pileus broadly conic with subacute to rounded apex, 13–23 mm broad, 7–16 mm tall, greyish brown (6E3–E4), hygrophanous to lighter greyish brown (5D2–D3) over disc, appearing smooth macroscopically; surface under hand lens a dense, very fine fibrillose mat, this uniform throughout with no erect elements evident, not translucent-striate; margin slightly inrolled, otherwise entire and light grey; moist. Lamellae thin, crowded, adnate with a decurrent tooth, 6.5–15 long, 1–2 mm tall, forking irregularly, white to light pink (6A1–A2); margins concolorous, highly roughened-cystidiate under hand lens; lamellulae numerous, too crowded to count. Stipe subequal, tapering very slightly toward apex, 74–87 mm × 3–4 mm (centrally), base 4–6 mm, concolorous with the pileus over upper ¾, smooth, finely longitudinally fibrillose under hand lens. Basal mycelium over basal ¼ of stipe a dense, white fibrillose mat. Pileus context light grey. Stipe context grey, hollow. Bruising reactions none. Odor faintly of detergent or chlorine; taste pleasantly fungoid.

Basidiospores distinctly (4–)5-angled and subisodiametric to heterodiametric in profile view, 6.6–8.4 × 5.1–7.2 µm (mean = 7.5 ± 0.4 × 6.0 ± 0.5 µm; Q = (1.09–)1.14–1.46, E = 1.26 ± 0.1; n = 25). Basidia clavate, strongly tapered basally, 27.6–35.6 × 5.8–11.1 µm (mean = 30.8 ± 2.3 × 8.5 ± 1.6 µm; Q = 2.95–5.1, E = 3.8 ± 0.8; n = 12); base 2.7–5.2 µm wide; 4–sterigmate; sterigmata 1.4–3.2 µm long. Cheilocystidia abundant, forming a sterile layer on the gill edge, uniformly cylindro-clavate, colorless, 47.9–74.3 × 3.7–5.9 µm. Pleurocystidia absent. Lamellar trama hyphae subparallel, relatively slender and uniform in width; cells 54.8–112.2 × 3.7–6.1 µm. Pileipellis a cuticular layer of largely repent, entangled, relatively narrow hyphae; terminal cells cylindro-clavate, 20.1–49.3 × 4.1–9.3 µm (n = 11). Pileal trama hyphae beneath the pileipellis slightly to distinctly inflated; cells 38.0–134.4 × 6.2–13.8 µm (n = 8), not measured elsewhere. Stipitipellis with an entangled hyphal layer similar to the pileipellis, with hymenial clusters at the stipe apex; terminal cells abundant, somewhat opaque, similarly-shaped as, but longer than the cheilocystidia, 20–137.8 × 3.2–6.3 µm (n = 7). Refractive hyphae abundant in the pileal trama, absent in the lamellar trama and the subhymenium. Pigmentation of two types in the pileipellis: uniformly cytoplasmic and faintly brownish and with external encrustations; pileus trama hyphae strongly externally encrusted. Clamp connections absent.

Ecology, range, distribution — Scattered on litter of forest floor in mixed *Dicymbe* spp. forest; known only from the type locality in the Upper Potaro River Basin of Guyana.

Comments — *Nolanea furcata* is distinguished by its conic, dark brownish grey, hygrophanous, opaque pileus, narrow, crowded, irregularly forked lamellae that are white initially, faintly detergent-like or chlorine-like odor,

abundant cylindro-clavate cheilocystidia and caulocystidia, cytoplasmic and externally incrusted pigment in the pileipellis, and relatively small (~7.5 × 6.0 µm) 4–5-angled basidiospores. *Entoloma infula* var. *chlorinosum* (Arnolds & Noordel.) Noordel. from Europe shares a dark brown pileus and stipe, the two types of pileipellis pigments, somewhat similarly shaped basidiospores, and chorine-like odor but fundamentally differs from *N. furcata* in its papillate pileus, persistent and much stronger chlorine-like odor, and lack of cheilocystidia (Noordeloos 1992). For additional information on *Nolanea* species similar to *N. furcata*, refer to the comments for *N. sinuolata*.

Nolanea mimiae Largent & Aime, **sp. nov.** PLATE 8

MYCOBANK MB 805595

Differs from *Nolanea mammosa* var. *venezuelana* by its non-papillate pileus and presence of pleurocystidia.

TYPE: Guyana. Region 8: Potaro-Siparuni. Pakaraima Mountains. Upper Potaro River Basin, ~15 km east of Mt. Ayanganna, environs of base camp located on Potaro River one km upstream from confluence with Whitewater Creek at 5°18′04.8″N 59°54′40.4″W, elevation 710–750 m; up Potaro River from base camp on line to Lance's plot near Blackwater Creek, 25 July 2010, Aime 4024 (BRG, **holotype**; PUL, **isotype**).

ETYMOLOGY: In honor of Mimi Chin, long-time Guyana field assistant and finder of the type collection.

PILEUS 19 mm broad, 11 mm tall, conical, lacking an umbo, glabrous macroscopically but faintly appressed-fibrillose under hand lens, opaque, not hygrophanous, not translucent striate, dark brown (6E7), slightly darker over the disc; margin crenulate and lighter. LAMELLAE subthin, close, adnate, 13 mm long, 3 mm tall, pale yellow (4A3) initially, later with pink spots; margins concolorous with face, roughened-cystidiate under hand lens. STIPE 60 mm × 1.5 mm, equal, dark yellowish brown (5F8), glabrous, inserted; trama hollow. BASAL MYCELIUM lacking. BRUISING REACTIONS none. ODOR indistinct; TASTE none at first, then somewhat soapy.

BASIDIOSPORES distinctly 5-angled, isodiametric to heterodiametric in profile view, 8.4–10.9 × 6.7–8.8 µm (mean = 9.8 ± 0.6 × 7.8 ± 0.5 µm; E = 1.1–1.5, Q = 1.26 ± 0.10; n = 34). BASIDIA cylindro-clavate, 27.6–32.0 × 10.5–13.5 µm (mean = 30.0 ± 1.5 × 11.7 ± 1.0 µm, E = 2.2–2.9, Q = 2.6 ± 0.3; n = 6), 4-sterigmate. CHEILOCYSTIDIA scattered to abundant, colorless, lageniform, 27.4–68.8 × 6.4–18.4 µm (E = 3.6–5.8; n = 9). PLEUROCYSTIDIA infrequent to scattered, similar in shape to cheilocystidia, 26.4–64.1 × 5.0–14.1 µm (E = 2.4–10.5; n = 14). LAMELLAR TRAMA HYPHAE subparallel; cells 187.4–480.6 × 8.6–29.5 µm (n = 4). PILEIPELLIS a cutis, 5–7 hyphal layers thick; hyphae 3.7–8.1 µm wide; TERMINAL CELLS cylindro-clavate, 26.9–37.1 × 2.5–7.6 µm (E = 4.4–10.8; n = 5). PILEAL TRAMA HYPHAE beneath the pileipellis slightly inflated, 5.7–14.3 µm wide; cells elsewhere inflated, relatively long and tapered

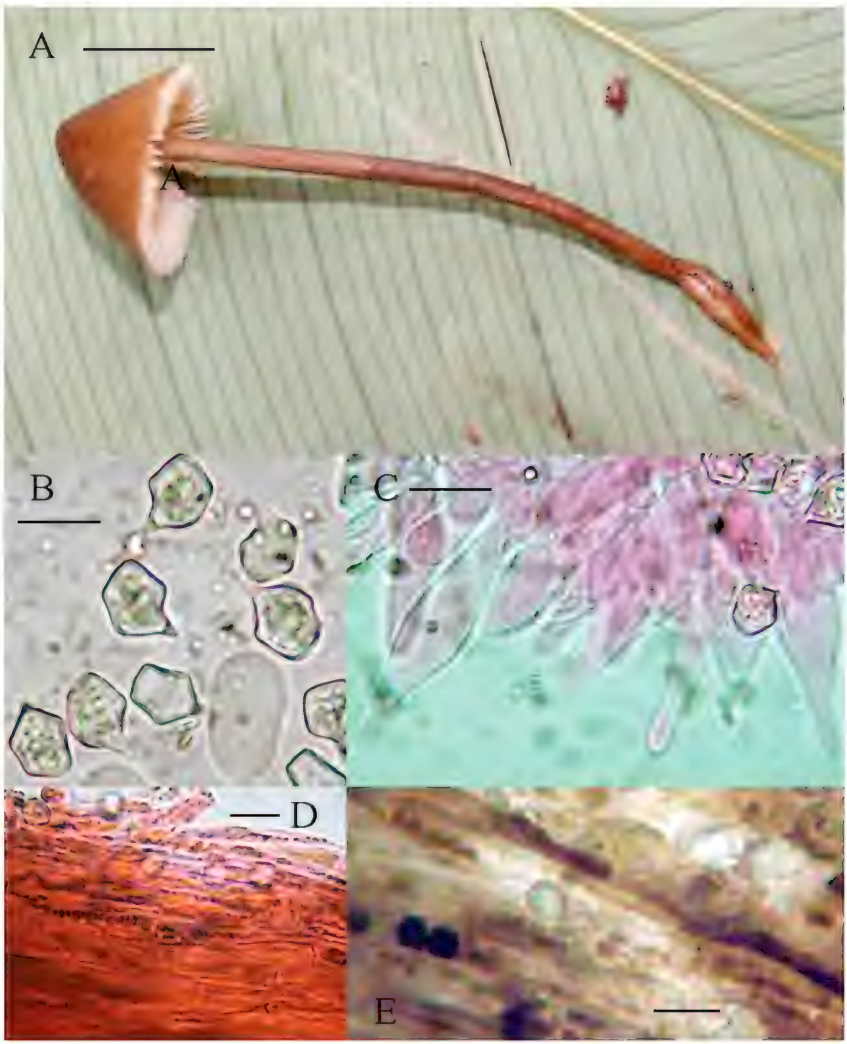


Plate 8. *Nolanea mimiae* (holotype; Aime 4024). A. Basidioma. B. Basidiospores. C. Cheilocystidia. D. Pileipellis. E. Cytoplasmic pigments in the pileipellis hyphae. Bars: A = 10 mm; B–E = 10 µm.

at both ends, 53.7–394.1 × 15.3–30.2 µm (n = 7). Stipitipellis at the apex with rare clusters of basidia, and rare terminal cells, these slightly out-turned, cylindro-clavate, 30.8–42.6 × 3.4–6.2 µm (n = 4), elsewhere a cutis. Stipe tramal hyphae 56.9–377.8 × 10.9–23.9 µm (n = 7). Refractive hyphae glistening in 3% KOH, abundant in the pileal trama, absent in the lamellar trama; not studied in the stipe trama. Pigmentation cytoplasmic in the hyphae

of the pileipellis, in the form of abundant dark brown, plaque-like agglutinated clots, absent in all other tissues. Clamp connections absent.

Ecology, range, distribution — Solitary on litter of forest floor in mixed *Dicymbe* spp. forest; known only from the type locality in the Upper Potaro River Basin of Guyana.

Comments — *Nolanea mimiae* is distinguished by its conical, non-umbonate, opaque, non-hygrophanous, dark brown pileus, pale yellow lamellae, dark yellowish brown stipe, scattered lageniform cheilocystidia and pleurocystidia, cytoplasmic pigment in the pileipellis, and nearly heterodiametric 5-angled basidiospores averaging 9.8 × 7.8 µm. These characters, along with the cytoplasmic pigment in the form of plaques or clots, and the mild then soapy taste, make *N. mimiae* unique among previously described *Nolanea* species.

Nolanea mammosa var. *venezuelana* Dennis from Venezuela [≡ *Rhodophyllus venezuelanus* (Dennis) Singer from Chile] and *Entoloma taedium* E. Horak from Chile are somewhat similar to *N. mimiae* because of their lageniform cheilocystidia and basidioma colors. *Nolanea mammosa* var. *venezuelana* differs from *N. mimiae* by its papillate pileus and lack of pleurocystidia (Singer 1969; Dennis 1970). *Entoloma taedium* differs by its distant pallid lamellae, lack of pleurocystidia, and cytoplasmic pigment in the cheilocystidia (Horak 1977).

Nolanea rava Largent & Aime, **sp. nov.** Plate 9

Mycobank MB 805596

Differs from *Entoloma acutopallidum* by its greyish yellow pileus, 5–6-angled basidiospores, obclavate to rostrate-ventricose cheilocystidia, and abundant pleurocystidia.

Type: Guyana. Region 10: Upper Demerara-Berbice. Mabura Ecological Reserve: ~100 m northwest of Mabura field station at 5°9′19.0″N 58°4′58.9″W, elevation 100 m; in *Dicymbe altsonii* monodominant stand #1, 27 May 2011, Aime 4312 (BRG, **holotype**; PUL, **isotype**).

Etymology: *ravus* (L. adj.) = greyish-yellow, referring to the color of the pileus and stipe.

Pileus 13 mm broad, 10 mm tall, conical with a tiny, nipple-like umbo that is 1 mm long, dry, silky, at first greyish yellow (4B4), remaining so on the disc and umbo, elsewhere becoming pale greyish yellow (4B3), hygrophanous, shallowly sulcate and translucent-striate to disc, glabrous; margin decurved, broadly undulating in outline. Lamellae thin, close, adnexed, 11.5 mm long, 3–5 mm tall, flesh-colored (6B3) with masses of concolorous basidiospores visible under hand lens; margins entire, concolorous and undulating; lamellulae 3–5 between lamellae. Stipe 37 × 2 mm at apex, 3 mm base, somewhat flattened and tapered from base to apex, glabrous but obscurely longitudinally striate under hand lens, greyish yellow (4B4) like the mature pileus disc. Basal mycelium absent.

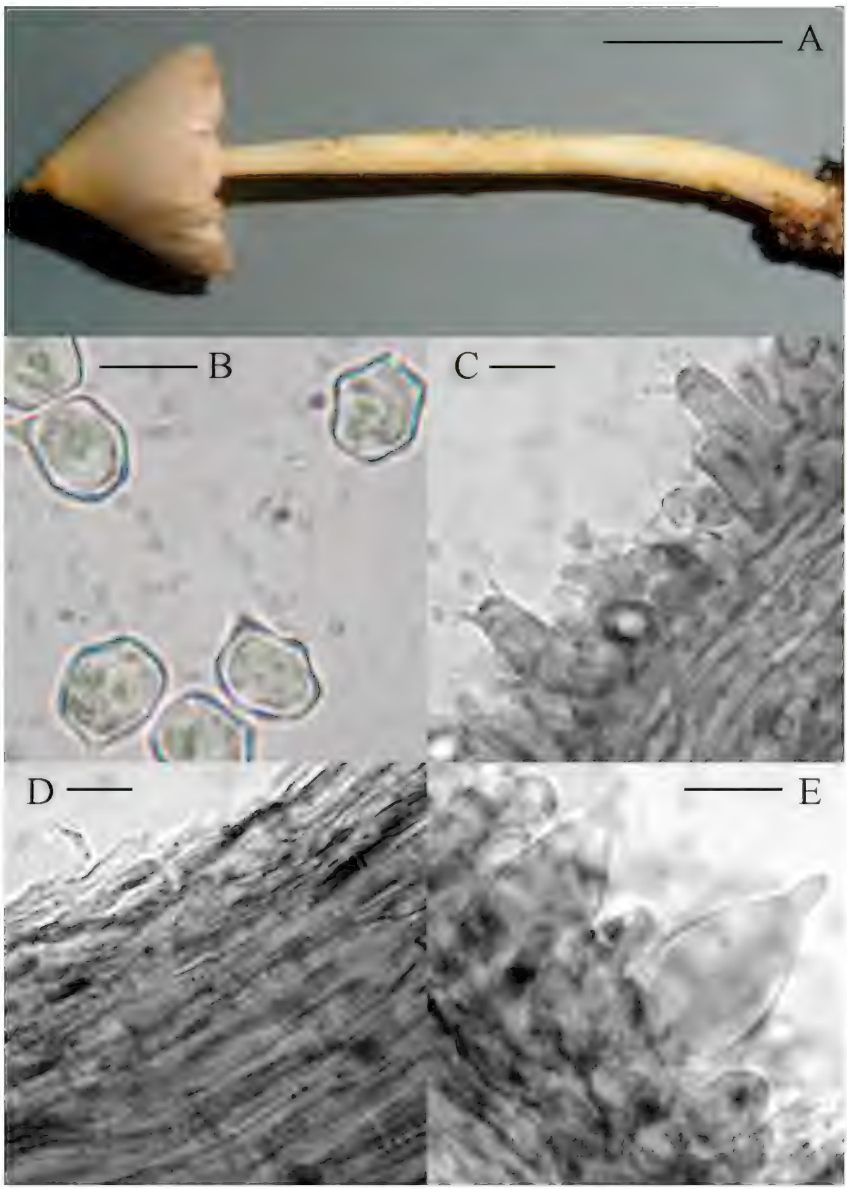


Plate 9. *Nolanea rava* (holotype; Aime 4312). A. Basidioma. B. Basidiospores. C. Hymenium with basidia. D. Pileipellis and pileal trama. E. Cheilocystidia. Bars: A = 10 mm; B–D = 10 µm.

Context not examined. Bruising reactions yellowish near the pileus margin. Odor none; taste not obtained.

Basidiospores distinctly 5–6-angled in all views, isodiametric to heterodiametric in profile and dorsiventral views, angles at times somewhat rounded, 9.4–11.9 × 7.6–9.8 µm (mean = 10.5 ± 0.6 × 8.5 ± 0.49 µm; E = 1.0–1.3, Q = 1.24 ± 0.08; n = 34). Basidia cylindro-clavate, hardly tapered, 29.8–43.0 × 10.5–13.5 µm (mean = 36.8 ± 3.7 × 11.6 ± 0.9 µm; E = 2.4–3.6, Q = 3.2 ± 0.4; n = 12); 4–sterigmate; sterigmata up to 5.0 µm long. Cheilocystidia abundant, colorless, obclavate to rostrate-ventricose, base rounded, 26.7–62.0 × 9.0–26.8 µm (mean = 42.7 ± 11.9 × 15.3 ± 6.1 µm; E = 1.9–4.9, Q = 3.0 ± 1.1; n = 8); rostrum 8.2–35.9 µm long × 4.3–11.6 µm wide at the base, 2.0–5.2 µm wide at the apex. Pleurocystidia abundant, similar in shape but longer than the cheilocystidia, colorless, 26.5–88.8 × 12.5–25.2 µm (mean = 49.8 ± 19.1 × 18.5 ± 4.2 µm, E = 1.6–3.4, Q = 2.6 ± 0.6; n = 11). Lamellar trama hyphae long and tapered at both ends; cells 53.4–498.7 × 8.3–25.4 µm (n = 9). Pileipellis a cutis, 2–3 hyphal layers thick; hyphae 4.0–7.5 µm wide; terminal cells cylindric and at times somewhat swollen at the apex. Pileal trama hyphae slightly inflated beneath the pileipellis, 10.3–14.3 µm wide; cells overall 44.2–270.5 × 3.4–14.3 µm (n = 6). Stipitipellis a cutis; hymenial elements and distinct terminal cells absent. Stipe trama hyphae not studied. Refractive hyphae absent. Pigmentation minimal, cytoplasmic in the pileipellis and stipitipellis. Clamp connections absent.

Ecology, range, distribution — Solitary on litter of forest floor in *Dicymbe altsonii* forests on sandy soils; known only from the type locality at Mabura Ecological Reserve, Guyana.

Comments — *Nolanea rava* is distinguished by its conical, acutely umbonate, silky, hygrophanous, translucent-striate greyish yellow pileus and concolorous stipe, nearly obclavate to rostrate-ventricose cheilocystidia and pleurocystidia, nearly heterodiametric 5–6-angled basidiospores averaging 10.5 × 8.5 µm, and general lack of microscopic pigmentation. This combination of characters makes *N. rava* unique among previously described *Nolanea* species.

The overall basidioma aspect of *Nolanea acuta* resembles that of *Entoloma acutopallidum* E. Horak & Cheype from French Guiana (Horak & Cheype 2008). *Entoloma acutopallidum* differs from *N. rava* by its orangish clay-colored pileus, white stipe, cuboid basidiospores, clavate to vesiculose cheilocystidia, and lack of pleurocystidia.

Nolanea sinuolata Largent, Aime & T.W. Henkel, **sp. nov.** Plate 10

Mycobank MB 805597

Differs from all described species of *Nolanea* by its nearly concolorous dark brown pileus, lamellae, and stipe, cylindro-clavate cheilocystidia and caulocystidia that are slightly wavy to sinuous in outline, and externally encrusted pigment on the hyphal walls of the pileipellis and lamellar trama.

Type: Guyana. Region 8: Potaro-Siparuni. Pakaraima Mountains. Upper Potaro River Basin, ~15 km east of Mt. Ayanganna, environs of base camp located on Potaro River one km upstream from confluence with Whitewater Creek at 5°18′04.8″N 59°54′40.4″W, elevation 710–750 m; 1 km NE of base camp, sandy zone E of Benny's Ridge, 29 August 2009, Henkel 8900 (BRG, **holotype**; HSU, **isotype**).

Etymology: *sinuolatus* (L. adj.) = faintly waved, referring to the cheilocystidia that are faintly wavy or sinuous in outline.

Pileus 11–24 mm broad, 5–12 mm tall, broadly conic with broadly rounded and flattened umbo, initially dark brown (6F4–6F5) throughout, then lighter concolorous (6E6 or 5E6) on the umbo and paling to light brown (5D6) toward the margin, glabrous macroscopically, under hand lens a contiguous mat with minute suberect elements over disc, moist, outer 1–2 mm of margin with faint translucent striations; marginal edge decurved, entire in areas, elsewhere very finely crenulate. Lamellae subthin, subcrowded, adnate, brown at first (concolorous with the pileus), then with pinkish overtones (5C4), finally dirty dull pink (6A2–6B2) with basidiospore maturation; margins whitish, densely cystidiate under hand lens; lamellulae 1–2 between lamellae, 0.5–3 mm long. Stipe 50–71 × 1.5–1.75 mm, equal, glabrous macroscopically, very finely twisting longitudinally striate under hand lens with a whitish basal bloom, otherwise dark yellowish brown (5F8) becoming dark brown (6F5–6F6) throughout, inserted, cartilaginous, hollow. Basal mycelium whitish and scant. Odor none or faintly spermatic; taste not obtained.

Basidiospores distinctly 5–6-angled in all views, in profile view isodiametric to heterodiametric, 6.7–9.5 × 5.3–7.9 µm (mean = 8.1 ± 0.6 × 6.8 ± 0.5 µm; E = 1.0–1.4, Q = 1.21 ± 0.1 subisodiametric; n = 47). Basidia cylindro-clavate and hardly tapered, rather narrow, 24.2–40.3 × 6.2–9.9 µm (mean = 34.0 ± 4.3 × 8.0 ± 1.12 µm; E = 3.2–5.8, Q = 4.33 ± 0.81; n = 17), 4–sterigmate. Cheilocystidia abundant, forming a sterile layer, colorless, narrowly cylindro-clavate and wavy to sinuous in outline, occasionally with a capitulate apex, 26.6–73.2 × 3.7–8.2 µm (mean = 49.7 ± 12.6 × 6.1 ± 1.18 µm; E = 3.9–12.8, Q = 7.23 ± 2.2; n = 22). Pleurocystidia absent. Lamellar trama subparallel to parallel, composed of broad hyphae, these 56–86 × 10–19 µm, along with narrow hyphae, these 83–85 × 7.2–7.4 µm (n = 9). Pileipellis a cutis but with a few loosely entangled clusters of cylindro-clavate terminal cells, semi–erect on the disc, repent elsewhere; terminal cells near disc 26.5–70.2 × 4.0–9.8 µm (mean = 43.8 ± 12.40 × 6.4 ± 2.22 µm; E = 4.61–14.32, Q = 7.41 ± 2.89; n = 12), near margin 51.2–88.2 × 4.0–7.0 µm (n/1 = 3). Pileal trama hyphae beneath the pileipellis rather short-celled and somewhat inflated; cells 38.9–138.8 × 7.0–17.9 µm (n = 9, not studied elsewhere). Stipitipellis with abundant clusters of caulocystidia, these similar in shape to the cheilocystidia; caulocystidia 28.8–77.9 × 3.4– 6.2 µm (mean = 45.5 ± 13.37 × 4.7 ± 0.99 µm; E = 5.0–13.5, Q = 9.9 ± 2.7; n = 12). Stipe trama hyphae broad in the center;

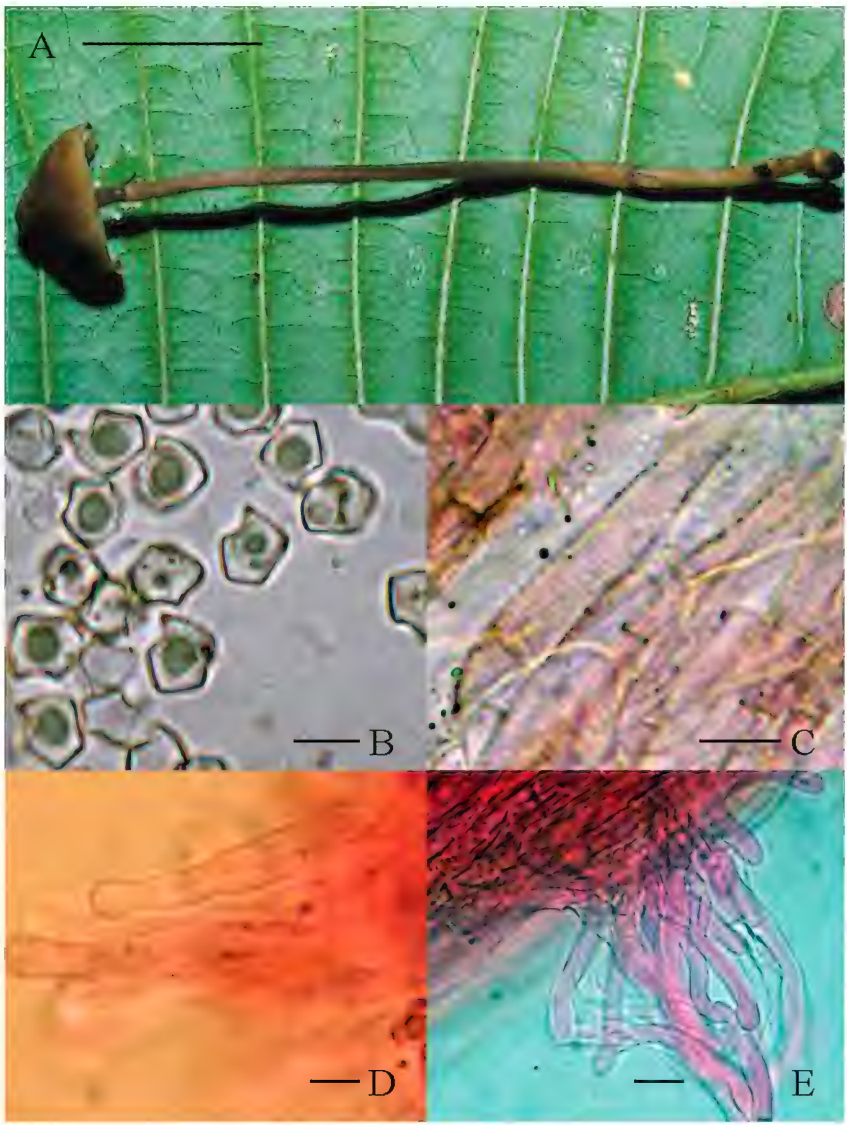


Plate 10. *Nolanea sinuolata* (holotype; Henkel 8900). A. Basidioma. B. Basidiospores. C. Pileus tramal hyphae with external encrustations. D. Cheilocystidia. E. Caulocystidia. Bars: A = 10 mm; B–E = 10 µm.

cells 81.0–228.0 × 13.5–20.3 µm (n = 5) and narrow near the stipitipellis; cells 168.3–223.2 × 3.0–3.5 µm (n = 5). Refractive hyphae absent. Pigmentation obvious and dark brown in 3% KOH in spot plates, dark brown in microscopic

sections, distinct and encrusting on the outer portions of the hyphae in the pileipellis, pileus trama, and lamellar trama. Clamp connections absent.

Ecology, range, distribution — Solitary on litter of forest floor in mixed *Dicymbe* spp. forest; known only from the Upper Potaro River Basin of Guyana.

Additional specimen examined. **GUYANA. Region 8: Potaro-Siparuni.** Pakaraima Mountains. Upper Potaro River Basin, ~15 km east of Mt. Ayanganna, environs of base camp located on Potaro River one km upstream from confluence with Whitewater Creek at 5°18′04.8″N 59°54′40.4″W, elevation 710–750 m; vicinity of base camp, 1 July 2003, Aime 2280 (BRG; PUL).

Comments — *Nolanea sinuolata* is diagnosed by its conic, broadly umbonate, dark brown pileus, brown, thin crowded lamellae, dark brown stipe, encrusted pigment on the pileus tramal hyphae, cylindro-clavate cheilocystidia with faintly wavy to sinuate sides, and 5–6-angled basidiospores averaging 8.1 × 6.8 µm. *Nolanea sinuolata*, *N. furcata*, *N. claviformis,* and *N. concentrica* (all described here as new) share the following features: a dark brown, conic or conic-campanulate, macroscopically glabrous but sericeous to faintly fibrillose pileus with a rounded, submammilate umbo; thin, close to crowded lamellae; a tough, cartilaginous stipe concolorous with the pileus; basidiospore shape; presence of cheilocystidia; an only slightly inflated subpellis; encrusted pigment on the outer hyphal walls of the pileipellis; and lack of clamp connections.

Among these species, *N. sinuolata* can be diagnosed by the brown lamellae when young, the encrusted pigment on lamellar tramal hyphae, cylindro-clavate cheilocystidia with slightly wavy walls and occasionally capitulate apex, the absence of cytoplasmic pigment in the pileipellis, and the lack of concentric ridges in the pileus. *Nolanea furcata* is distinguished by the white lamellae that fork toward the margin, the abundant tomentum covering the basal ¼ of the clavate stipe, the chlorine odor, and a cytoplasmic pileipellis pigment. *Nolanea claviformis* and *N. concentrica* have a concentrically ridged or beveled pileus, white lamellae, and also have a cytoplasmic pigment in the hyphae of the pileipellis. *Nolanea claviformis* is distinguished from *N. concentrica* by the clavate cheilocystidia, larger (~9.0 × 7.5 µm) basidiospores, and larger (28.3–42 5 × 9.5–12.0 µm) basidia. *Nolanea concentrica* has aciculate to rostrate-ventricose cheilocystidia, smaller (~7.7 × 6.0 µm) basidiospores, and smaller (24.2–30.6 × 6.7–9.5 µm) basidia.

Nolanea subsulcata Largent & T.W. Henkel, **sp. nov.** Plate 11

Mycobank MB 519977

Differs from *Entoloma guatopoanum* by its glabrous pileus and stipe.

Type: Guyana. Region 8: Potaro-Siparuni. Pakaraima Mountains. Upper Potaro River Basin, ~15 km east of Mt. Ayanganna, environs of base camp located on Potaro River one km upstream from confluence with Whitewater Creek at 5°18′04.8″N 59°54′40.4″W, elevation 710–750 m; vicinity of base camp in *Dicymbe* forest, 1 June 2005, Henkel 8825 (BRG, **holotype**; HSU, **isotype**).

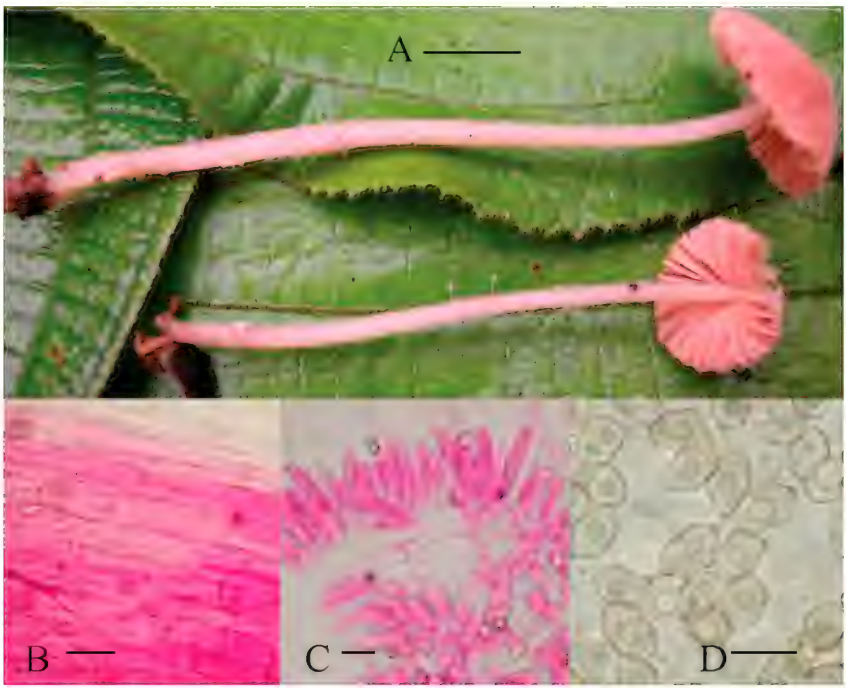


Plate 11. *Nolanea subsulcata* (holotype; Henkel 8825). A. Basidiomata. B. Pileipellis. C. Basidia. D. Basidiospores. Bars: A = 10 mm; B–D = 10 µm

Etymology: *sub–* and *sulcatus* (L. adj.) = somewhat grooved, referring to the pileus surface texture.

Pileus 16–22 mm broad, 5–10 mm tall, broadly conic with low, rounded umbo, brownish orange (6C4 "red-haired"–7C4) throughout, hygrophanous over disc with age, striate to subsulcate over outer 4/5, more smooth over immediate disc; surface glabrous and nearly translucent when fresh, under hand lens appearing finely longitudinally appressed-fibrillose, disc with no distinct vestiture, moist; margin finely and irregularly crenulate at sulcations; pileus trama extremely thin, less than 1 mm throughout. Lamellae subthin, subdistant, adnexed, brownish orange (7C4); margins concolorous, smooth; lamellulae 1 between lamellae, 1–2 mm long. Stipe 56–80 × 2–2.5 mm, equal, light greyish orange (6B3 "flesh"–6B4), appearing smooth but with fine appressed longitudinal fibrils under hand lens, cartilaginous. Basal mycelium absent. Odor and taste not noted.

Basidiospores distinctly 6–8-angled in profile, dorsal, and ventral views, sides slightly concave, heterodiametric, 8.2–12.3 × 5.1–7.2 µm, (mean = 9.7 ±

0.9 × 6.4 ± 0.51 µm; E = 1.3–1.8, Q = 1.5 ± 0.14; n = 31). Basidia subcylindric to subclavate, barely tapering toward base, 26.9–37.3 × 5.7–10.6 µm, (mean = 30.0 ± 3.0 × 8.3 ± 1.2 µm; E = 2.7–4.8, Q = 3.7 ± 0.63; n = 13), 2 or 4-sterigmate. Cheilocystidia and pleurocystidia absent. Lamellar trama subparallel, composed of broad, long-celled hyphae (not measured). Pileipellis a cutis of 1–3 uninflated hyphal layers; hyphae 3.2–10.0 µm wide; terminal cells cylindric to cylindro–clavate, 31.0–74.2 µm long. Pileus trama hyphae beneath the pileipellis moderately inflated, 5–18 µm wide, elsewhere 5-40 µm wide. Stipitipellis a cutis, lacking hymenial clusters or caulocystidia. Refractive hyphae abundant in the pileus trama, scattered in the lamellar trama. Pigmentation cytoplasmic, faintly evident in 3% KOH. Clamp connections absent.

Ecology, range, distribution — Solitary on humic litter layer of forest floor in *Dicymbe corymbosa* forest; known only from type locality in the Upper Potaro River Basin in Guyana.

Comments — *Nolanea subsulcata* is distinguished by its broadly conic, umbonate, subsulcate, translucent-striate, hygrophanous pileus with a paler crenulate margin; equal stipe; overall brownish orange to flesh colors throughout; lack of hymenial cystidia and clamp connections; and 6–8-angled, heterodiametric basidiospores averaging 9.7 × 6.4 µm. *Entoloma guatopoanum* (Dennis) E. Horak [≡ *Leptonia guatopoana* Dennis] from Venezuela, which also has a sulcate-striate similarly colored pileus and heterodiametric basidiospores measuring 9–11 × 6–8 µm and lacks hymenial cystidia and clamp connections, can be distinguished from *N. subsulcata* by its pruinose pileus and its white, minutely pruinose stipe with hyaline downy hairs (Horak 1977). *Rhodophyllus lutensis* Romagn. & Gilles from Gabon has similarly sized, shaped, and colored basidiomata but differs from *N. subsulcata* in its non-hygrophanous, non-striate pileus, and much smaller (7–8.2 × 5–6 µm) basidiospores (Romagnesi & Gilles 1979).

Acknowledgments

This research was made possible by grants from the National Geographic Society's Committee for Research and Exploration and NSF DEB-0918591 to TWH, and an Explorer's Club Exploration and Field Research Grant to MCA. Dillon Husbands functioned as Guyanese local counterpart and assisted with field collecting, descriptions, and specimen processing. Mimi Chin, C. Andrew, L. Williams, V. Joseph, F. Edmund, and L. Edmund provided field assistance in Guyana. Research permits were granted by the Guyana Environmental Protection Agency. Our special thanks go to Drs. Ron Petersen and Clark Ovrebo for their critical comments and revisions, and Dr. Shaun Pennycook for nomenclatural edits. This paper is number 206 in the Smithsonian Institution's Biological Diversity of the Guiana Shield Program publication series.

Literature cited

Baroni TJ, Matheny PB. 2011. A re-evaluation of gasteroid and cyphelloid species of *Entolomataceae* from Eastern North America. Harvard Papers in Botany 16: 293–310. http://dx.doi.org/10.3100/0.25.016.0205

Co-David D, Langeveld D, Noordeloos ME. 2009. Molecular phylogeny and spore evolution of *Entolomataceae*. Persoonia 23: 147–176. http://dx.doi.org/10.3767/003158509X480944

Dennis RWG. 1953. Les Agaricales de l'Ile de la Trinité. *Rhodosporae-Ochrosporae*. Bulletin de la Société Mycologique de France 69: 145–198.

Dennis RWG. 1970. Fungus flora of Venezuela and adjacent countries. Kew Bulletin Additional Series 3: 1–531.

Fries E. 1821. Systema mycologicum, vol. 1. Lundae: Ex Officina Berlingiana.

He XL, Li TH, Xi PG, Jiang ZD, Shen YH. 2013. Phylogeny of *Entoloma* s.l. subgenus *Pouzarella*, with descriptions of five new species from China. Fungal Diversity 58: 227–243. http://dx.doi.org/10.1007/s13225-012-0212-7

Henkel TW. 2003. Monodominance in the ectomycorrhizal *Dicymbe corymbosa* (*Caesalpiniaceae*) in Guyana. Journal of Tropical Ecology 19: 417–437. http://dx.doi.org/10.1017/S0266467403003468

Hesler LR. 1967. *Entoloma* in southeastern North America. Beihefte zur Nova Hedwigia 23: 1–196.

Holmgren PK, Holmgren NH, Barnett LC. 1990. Index Herbariorum I: the herbaria of the world. Regnum Vegetabile 120: 1–693.

Horak E. 1977. *Entoloma* in South America I. Sydowia 30: 40–111.

Horak E. 1980. *Entoloma* (*Agaricales*) in Indomalaya and Australasia. Beihefte zur Nova Hedwigia 65: 1–352.

Horak E, Cheype J-L. 2008. *Entoloma acutopallidum* and *Entoloma pileofibrosum:* Two new species from French Guiana. Bulletin de la Société Mycologique de France 124: 287–298.

Horak E, Desjardin DE. 1993. *Agaricales* of the Hawaiian Islands. 2. Notes on some *Entoloma* species. Mycologia 85: 480–489. http://dx.doi.org/10.2307/3760708

Kornerup A, Wanscher JH. 1978. Methuen handbook of colour. 3rd ed. Chichester, Richard Clay Ltd.

Largent DL. 1994. Entolomatoid Fungi of the Western United States and Alaska. Eureka, Mad River Press Inc.

Largent DL, Baroni TJ. 1988. How to identify mushrooms to genus VI: modern genera. Eureka, Mad River Press Inc.

Largent DL, Johnson D, Watling R. 1977. How to identify mushrooms to genus III: microscopic features. Eureka, Mad River Press Inc.

Largent DL, Henkel TW, Aime MC, Baroni TJ. 2008. The Entolomataceae of the Pakaraima Mountains of Guyana I: four new species of *Entoloma* s. str. Mycologia 100: 132–140. http://dx.doi.org/10.3852/mycologia.100.1.132

Murrill, WA. 1911. The Agaricaceae of tropical North America IV. Mycologia 3: 271–282. http://dx.doi.org/10.2307/3753496

Noordeloos ME. 1980. *Entoloma* subgenus *Nolanea* in the Netherlands and adjacent regions with a reconnaissance of its remaining taxa in Europe. Persoonia 10: 427–534.

Noordeloos ME. 1988. *Entoloma* in North America. The species described by L.R. Hesler, A.H. Smith, and S.J. Mazzer. Cryptogamic Studies 2: 1–164.

Noordeloos ME. 1992. *Entoloma* s. l. Fungi Europaei 5: 1–760.

Noordeloos ME. 2004. *Entoloma* s. l. Fungi Europaei 5A: 761–1378.

Pegler DN. 1983. Agaric flora of the Lesser Antilles. Kew Bulletin Additional Series 9: 1–668.

Pegler DN. 1997. The agarics of São Paulo, Brazil. London, Royal Botanic Garden Kew.

Romagnesi H. 1941. Les Rhodophylles de Madagascar (*Entoloma, Nolanea, Leptonia, Eccilia, Claudopus*). Prodrome á une Flore Mycologique de Madagascar 2: 1–164.

Romagnesi H, Gilles G. 1979. Les Rhodophylles des forêts côtières du Gabon et de la Côte d'Ivoire avec une introduction générale sur la taxonomie du genre. Beihefte zur Nova Hedwigia 59: 1–649.

Singer R. 1969. Mycoflora australis. Beihefte zur Nova Hedwigia 29: 1–405.

ISSN (print) 0093-4666 © 2014. Mycotaxon, Ltd. ISSN (online) 2154-8889

MYCOTAXON

http://dx.doi.org/10.5248/129.149

Volume 129(1), pp. 149–152 *July–September 2014*

Two new species of *Xylaria* and *X. diminuta* new to China

Gu Huang[1, 2] Lin Guo[1*], & Na Liu[2]

[1]*State Key Laboratory of Mycology, Institute of Microbiology, Chinese Academy of Sciences, Beijing 100101, China*

[2]*Henan University of Technology, College of Biological Engineering, Zhengzhou 450001, China*

* Correspondence to: *guol@im.ac.cn*

Abstract —Two new species, *Xylaria beilschmiediae* on fallen fruit of *Beilschmiedia percoriacea* (*Lauraceae*) and *Xylaria foliicola* on dead leaves, are described. *Xylaria diminuta* is new to China. All specimens were collected from Yunnan Province in China.

Key words —*Ascomycota,* pyrenomycetous fungi, *Xylariaceae,* taxonomy

Four fruit-inhabiting *Xylaria* species have previously been recorded in China: *X. byttneriae* G. Huang et al., *X. culleniae* Berk. & Broome, *X. ianthinovelutina* (Mont.) Fr., and *X. liquidambaris* J.D. Rogers et al. (Teng 1963, Tai 1979, Rogers 1979, Huang et al. 2014). In this paper we describe two new *Xylaria* species and report *X. diminuta* as a new record from China.

Xylaria beilschmiediae G. Huang & L. Guo, **sp. nov.** Figs 1–3

Fungal Name FN570101

Differs from *Xylaria himalayensis* in its perithecia immersed within a stroma, overlain with dark brown outer peeling layer.

Type: China, Yunnan, Jinghong, Guanping, alt. 870 m, on fallen fruit of *Beilschmiedia percoriacea* C.K. Allen (*Lauraceae*), 18.X.2013, G. Huang, L. Guo & W. Li 285 (HMAS 269888, **holotype**).

Etymology: From *Beilschmiedia,* the genus of the plant serving as a substrate.

Stromata cylindrical, unbranched, with a sterile apex, 12–25 × 1–2 mm, fertile parts 3–10 mm long, on pubescent stipes; surface blackish, immersed perithecia, overlain with dark brown outer peeling layer, with perithecial mounds; interior white. Perithecia subglobose or ellipsoid, 330–500 µm diam; ostioles papillate. Asci with eight ascospores arranged in uniseriate manner, cylindrical, 138–165 µm total length, 6–8 µm broad, the spore-bearing parts 75–88 µm long, with an

apical ring staining blue in Melzer's iodine reagent, hat-shaped, 3–4 µm high, 2–3 µm broad. Ascospores brownish or brown, ellipsoid-inequilateral, smooth, (11–)12–14 × 4–5(–6) µm, with straight germ slit much less than spore-length.

Comments: *Xylaria beilschmiediae* is similar to *X. himalayensis* Narula & Rawla, which differs in producing stromata with protruding perithecia, in lacking an outer peeling layer, and which occurs on dead angiosperm fruits (Narula et al. 1985).

Xylaria foliicola G. Huang & L. Guo, **sp. nov.** Figs 4–6

Fungal Name FN570102

Differs from *Xylaria hainanensis* in its vertically cracked stromal surface and hairless plicate stipes.

Type: China, Yunnan, Jinghong, Dadugang, alt. 1250 m, on dead leaves, 20.X.2013, G. Huang, L. Guo & W. Li 259 (HMAS 253028, **holotype**).

Etymology: The epithet refers to the substrate (leaves) that the fungus inhabits.

Stromata cylindrical, unbranched, occasional branched, with a sterile greyish apex, overall 23–35 mm × 1–2 mm, fertile parts 7–18 mm long, on plicate stipes; surface black, overlain with outer peeling layer, with perithecial mounds; interior white. Perithecia subglobose or ellipsoid, 400–650 µm diam; ostioles papillate. Asci with eight ascospores arranged in uniseriate manner, cylindrical, 120–137 µm total length, 5–9 µm broad, the spore-bearing parts 56–68 µm long, with an apical ring staining blue in Melzer's iodine reagent, inverted hat-shaped, 2–3 µm high, 1.5–2.5 µm broad. Ascospores brownish or brown, ellipsoid–inequilateral, smooth, (8.5–)9–11 × 4–6 µm, with straight germ slit along the length of the spore.

Comments: *Xylaria foliicola* is similar to *X. hainanensis* Y.F. Zhu & L. Guo, which differs in having smooth stromata without vertical cracks on the surface, tomentose stipes, shorter asci (65–70 µm overall with 35–45 µm long spore-bearing parts; Zhu & Guo 2011).

Xylaria diminuta F. San Martín & J.D. Rogers, Rev. Mex. Micol. 13: 63, 1998. Figs 7–8

Stromata cylindrical, unbranched (occasionally branched), with a sterile apex, overall 12–40 mm × 0.8–1 mm, fertile parts 12–14 mm long, on plicate stipes; surface black, smooth, with perithecial mounds; interior white. Perithecia subglobose or ellipsoid, 270–430 µm diam; ostioles papillate. Asci

---

Figs 1–3. ***Xylaria beilschmiediae*** (HMAS 269888, holotype). 1. Stromata on fruit; 2. Stromatal surface; 3. Asci and ascospores. Figs 4–6. ***Xylaria foliicola*** (HMAS 253028, holotype). 4. Stromata on dead leaf; 5. Stromatal surface; 6. Asci and ascospores. Figs 7–8. ***Xylaria diminuta*** (HMAS 269887). 7. Stroma on dead leaf; 8åœ. Asci and ascospores.

1
2
3
10 µm
4
5
6
10 µm
7
8
10 µm

with eight ascospores arranged in uniseriate manner, cylindrical, 73–82 µm total length, 4–6 µm broad, the spore-bearing parts 32–45 µm long, with an apical ring staining blue in Melzer's iodine reagent, rectangle, 1.5–2 µm high, 1–1.5 µm broad. Ascospores brownish or brown, ellipsoid–inequilateral, smooth, (5.5–)6–8 × 3–3.5(–4) µm, with straight germ slit spore-length.

Specimen examined: CHINA, Yunnan, Jinghong, Dadugang, alt. 1250 m, on dead leaves, 20.X.2013, G. Huang, L. Guo & W. Li 254 (HMAS 269887).

Remarks: The Chinese specimen agrees morphologically with the original description of *Xylaria diminuta*, except that the type specimen has shorter (5–13 mm) stromata and a shorter apical ring (1–1.3 µm long; San Martín et al. 1998).

Acknowledgements

The authors would like to express their deep thanks to Prof. Anthony J.S. Whalley (Liverpool, UK) and Dr. L. Vasilyeva (Vladivostok, Russia) for serving as pre-submission reviewers, to Dr. Shaun Pennycook (Auckland, New Zealand) for nomenclatural review, and to Prof. Zhenyu Li (Institute of Botany, Chinese Academy of Sciences) for identifying the host plant. This study was supported by the foundation of Project of the Knowledge Innovation Program of the Chinese Academy of Sciences (No. KSCX2-EW-Z-9).

Literature cited

Huang G, Guo L, Liu N. 2014. *Xylaria byttneriae* sp. nov. from Yunnan Province in China. Mycosystema 33: 567–570.

Narula AM, Rawla GS, Kaushal SC. 1985 ["1984"]. Two new species of *Xylaria* (*Pyrenomycetes*) from India. Willdenowia 14(2): 409–411.

Rogers JD. 1979. *Xylaria magnoliae* sp. nov. and comments on several other fruit-inhabiting species. Can. J. Bot. 57: 941–945. http://dx.doi.org/10.1139/b79-115

San Martin, Rogers JD, Lavin P. 1997. Algunas especies de *Xylaria* (*Pyrenomycetes, Sphaeriales*) habitantes en hojarasca de bosques mexicanos. Rev. Mex. Micol. 13: 58–69.

Tai FL. 1979. Sylloge Fungorum Sinicorum. Science Press, Beijing. 1527 p.

Teng SC. 1963. Fungi of China. Science Press, Beijing. 808 p.

Zhu YF, Guo L. 2011. *Xylaria hainanensis* sp. nov. (*Xylariaceae*) from China. Mycosystema 30: 526–528.

ISSN (print) 0093-4666 © 2014. Mycotaxon, Ltd. ISSN (online) 2154-8889

MYCOTAXON

http://dx.doi.org/10.5248/129.153

Volume 129(1), pp. 153–161 *July–September 2014*

Erysiphe magnoliicola, a new powdery mildew on *Magnolia*

Sung-Eun Cho[1], Susumu Takamatsu[2], Jamjan Meeboon[2, 3], & Hyeon-Dong Shin[1*]

[1]*Division of Environmental Science and Ecological Engineering, Korea University, Seoul, 136-701, Korea*

[2]*Department of Bioresources, Graduate School, Mie University, Tsu 514-8507, Japan*

[3]*Department of Agriculture, 50 Phaholyothin Rd., Ladyao, Chatuchak, Bangkok 10900, Thailand*

*Correspondence to: *hdshin@korea.ac.kr*

Abstract — A new powdery mildew species of *Erysiphe* sect. *Microsphaera*, collected on three species of *Magnolia*, is here described, illustrated, and named as *E. magnoliicola* sp. nov. Introduction of a new species is supported by phylogenetic analyses of ITS and 28S rDNA of Korean and Japanese collections of this fungus. A comparison of and a synoptic key to the four species of *Erysiphe* sect. *Microsphaera* known on *Magnolia* spp. are provided.

Key words — *Erysiphaceae, Erysiphe magnifica, Magnoliaceae*, molecular phylogeny

Introduction

Powdery mildews (*Erysiphaceae*, *Ascomycota*) are plant pathogenic fungi that are holobiotrophic parasites on leaves, stems, flowers, and fruits. These pathogens cause moderate to serious damage on nearly 10,000 species of angiosperms (Braun et al. 2002, Glawe 2008). Braun (1987) introduced a classification of the *Erysiphaceae* that split the family into 18 genera, including *Erysiphe*, *Microsphaera*, and *Uncinula*. Later Braun & Takamatsu (2000) reduced the genera *Microsphaera* and *Uncinula* to synonymy with *Erysiphe* s. lat. based on phylogenetic analyses and proposed two new non-phylogenetic sections: *E.* sect. *Uncinula* (Lév.) U. Braun & Shishkoff and *E.* sect. *Microsphaera* are characterized by having chasmothecial appendages with dichotomously branched tips.

Three species of *Erysiphe* sect. *Microsphaera* — *E. magnifica* (U. Braun) U. Braun & S. Takam., *E. magnoliae* (Sawada) U. Braun & S. Takam., and *E. bulbosa* (U. Braun) U. Braun & S. Takam. — have been recorded on *Magnolia* spp. *Erysiphe magnifica* is a well-known species infecting *Magnolia* species in North and South America, eastern Asia, and Europe (Braun 1987 & 1995,

Glawe 2003, Braun & Cook 2012, Farr & Rossman 2014). *Erysiphe magnoliae* is known from Japan and Korea as the causal agent of powdery mildew on *M. obovata* (Nomura 1997, Braun & Cook 2012, Takamatsu et al. 2013). This species is clearly distinguished from *E. magnifica* by having conidiophore foot-cells with inflated bases, and the status of *E. magnoliae* as a separate species has recently been confirmed by molecular data (Takamatsu et al. 2013). *Erysiphe bulbosa*, endemic to China, was recorded on *M. liliiflora* (Braun 1988; Wang 1987). This fungus can be differentiated from *E. magnifica* and *E. magnoliae* by having chasmothecial appendages with a distinct swelling at the base.

During a field survey of phytopathogenic fungi carried out after the publication of comprehensive descriptions of powdery mildews in Korea (Shin 1988, 2000), a new powdery mildew species was found on *Magnolia* species. In a previous study, it was suggested that *E. magnoliae* has unique characteristics compared to other powdery mildew species described on *Magnolia* (Takamatsu et al. 2013). However, this study, which focused on *E. magnoliae,* was not a comprehensive study of *Magnolia* powdery mildews.

We describe our new species found in Korean and Japanese specimens based on morphological characters and molecular analyses and discuss relationships with closely related powdery mildew fungi infecting *Magnolia* species.

Materials & methods

The fungus was detached from the infected leaves and mounted in a few drops of distilled water on a glass slide for light microscopy. The morphology of the fungal structures was examined in bright field and differential interference contrast (DIC) light microscopy, using an Olympus BX51 microscope (Olympus, Tokyo, Japan) for measurements and a Zeiss AX10 microscope equipped with AxioCam MRc5 (Carl Zeiss, Göttingen, Germany) for photographs. The measurements were performed at 100×, 200×, 400×, and 1000× magnifications. Voucher specimens were deposited in the Korea University Herbarium (KUS), Korea; and the Mie University Mycological Herbarium (MUMH), Japan.

Genomic DNA was extracted from chasmothecia using the Chelex 100 as previously described (Walsh et al. 1991, Hirata & Takamatsu 1996). The 28S rDNA (including domains D1 and D2) and internal transcribed spacer (ITS) rDNA (ITS1, 5.8S, ITS2) regions were amplified by the polymerase chain reaction (PCR), and then sequenced directly (Takamatsu et al. 2009). The sequences were edited using the DNASTAR computer package version 5.05 (Lasergene, Madison, WI, USA) and resulting sequences were deposited in GenBank under the accession numbers of KJ567065–KJ567075. Additional sequences of *Erysiphe* spp. were retrieved from GenBank. Alignments were done with an online version of MAFFT 7 (Katoh & Standley 2013). Maximum likelihood analyses were performed using PhyML 3.0 (Guindon & Gascuel 2003). The best fitting substitution models were determined with jModelTest 2.1.4 (Darriba et al. 2012). The branches of the inferred tree were tested by the bootstrap analysis of 1000 replicates. The phylogenetic tree generated from the ITS analysis was deposited in TreeBASE (http://www.treebase.org/) under the accession number of S15516.

Taxonomy

Erysiphe magnoliicola S.E. Cho, S. Takam. & H.D. Shin, **sp. nov.** FIG. 1

MYCOBANK MB 808247

Differs from *Erysiphe magnifica* by its longer conidiophore foot-cells and its smaller number of chasmothecial appendages and from *E. magnoliae* by its conidiophore foot-cells with a cylindrical base.

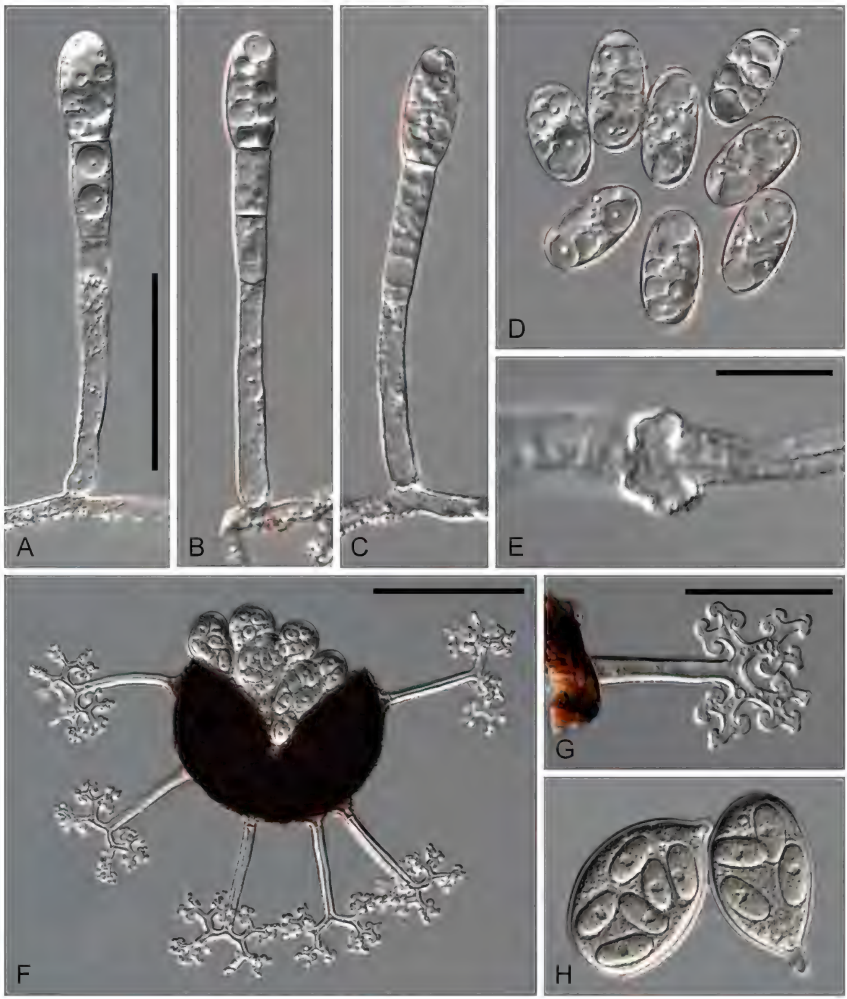


FIG. 1. *Erysiphe magnoliicola*. A–C: Conidiophores. D: Conidia. E: Appressoria. F: Surface view of a conidium showing angular/rectangular wrinkling pattern. G: Chasmothecium containing several asci. H: Appendage. I: Asci containing 4–6 ascospores. Scale bars: A–D, H–I = 50 µm; E–F = 10 µm; G = 100 µm.

Type: 38°11′31″N 128°21′44″E, on *Magnolia sieboldii* K.Koch (*Magnoliaceae*), 24 September 2012, H.D. Shin & S.E. Cho (**Holotype**, KUS-F26977; GenBank KJ567072, KJ567065).

Etymology: The epithet is derived from the host plant genus.

Colonies amphigenous, thinly effused or conspicuous, forming circular to irregular white patches. Hyphae straight or almost so, hyaline, septate, branched, 4–8 µm wide. Hyphal appressoria well developed, moderately lobed to multi-lobed, solitary or in opposite pairs. Conidiophores arising from the upper part of mother cells, 85–137 × 8.5–10 µm, producing conidia singly, followed by 2 to 3 cells. Foot-cells mostly straight or almost so, slightly sinuous at the base, relatively long, 42.5–70 µm. Conidia formed singly, hyaline, ellipsoidal to oval, 32.5–42.5 × 17.5–21.3 µm with a length/width ratio of 1.6–2.5, lacking fibrosin bodies, producing germ tubes on perihilar position, with an angular/rectangular wrinkling pattern on the surface. Chasmothecia amphigenous, mostly hypophyllous, cauligenous, gregarious, dark brown, spherical, 100–140 µm diam., containing 4–7 asci. Peridium cells irregularly polygonal or rounded, 10–25 µm wide. Appendages equatorial, 6–10, mostly aseptate, rarely 1-septate, 4–5 times regularly dichotomously branched, 0.5–1 times as long as the chasmothecial diam., 70–100 × 7.5–9 µm, tips distinctly recurved, brown at the base, becoming paler toward the tip. Asci obovoid to ellipsoid or saccate, mostly short-stalked, 57.5–70 × 40–45 µm, containing 3–6 spores per ascus. Ascospores hyaline, oblong-ovoid, 20–27.5 × 10–12.5 µm.

Additional specimens examined –

On *Magnolia sieboldii*: **KOREA**, **Pyeongchang**, Byeongnae-ri, 13 Sep. 1999, H.D. Shin (KUS-F16655); **Hongcheon**, Bukbang-myeon, 29 Sep. 2000, H.D. Shin (KUS-F17710); 17 Sep. 2001, H.D. Shin (KUS-F18474); 11 Oct. 2002, H.D. Shin (KUS-F19252); 9 Sep. 2007, H.D. Shin (KUS-F22848); Nae-myeon, 20 Aug. 2012, H.D Shin & S.E. Cho (KUS-F26833); **Suwon**, Seodun-dong, 5 Nov. 2004, H.D. Shin (KUS-F20960); 20 Sep. 2005, H.D. Shin (KUS-F21395); **Namyangju**, Experimental Farm of Korea University, 19 Oct. 2005, H.D. Shin (KUS-F21546; GenBank KJ567072, KJ567065); Sudong-myeon, 13 Aug. 2012, H.D. Shin & S.E. Cho (KUS-F26807); **Gangneung**, Seongsan-myeon, 29 Aug. 2011, H.D. Shin & S.E. Cho (KUS-F26035); 3 Oct. 2012, H.D. Shin & S.E. Cho (KUS-F27041); **Gapyeong**, Seolak-myeon, 23 Aug. 2012, H.D. Shin & S.E. Cho (KUS-F26843); **Inje**, Yongdae-ri, 24 Sep. 2012, H.D. Shin & S.E. Cho (KUS-F26977); **Gyeongju**, Forest Environment Research Institute, 8 Oct. 2012, H.D. Shin & S.E. Cho (KUS-F27077).

On *Magnolia liliiflora* Desr.: **KOREA**, **Suwon**, Seodun-dong, 17 Oct. 1997, H.D. Shin (KUS-F14447); 4 Oct. 1999, H.D. Shin (KUS-F16918); 16 Oct. 2008, H.D. Shin (KUS-F23831); 19 Oct. 2011, H.D. Shin & S.E. Cho (KUS-F26353); 14 Aug. 2012, H.D. Shin & S.E. Cho (KUS-F26821); 11 Oct. 2012, H.D. Shin & S.E. Cho (KUS-F27100); **Jinju**, Gajwa-dong, 15 Oct. 2003, H.D. Shin (KUS-F19903; GenBank KJ567074, KJ567069); 1 July 2004, H.D. Shin (KUS-F20383); **Jeju**, Jeju-si, 13 Nov. 2003, H.D. Shin

(KUS-F20074); 23 Oct. 2012, H.D. Shin & S.E. Cho (KUS-F27154); **Gyeongju**, Forest Environment Research Institute, 17 Jun. 2011, H.D. Shin (KUS-F25786); **Seoul**, Korea Forest Research Institute, 13 Oct. 2012, H.D. Shin & S.E. Cho (KUS-F27111; GenBank KJ567073, KJ567067).

On *Magnolia denudata* Desr.: **JAPAN, Gifu Prefecture**, Sekigahara-cho, 8 Nov. 2011, S. Takamatsu & J. Meeboon (MUMH5501; GenBank KJ567075, KJ567068).

Phylogenetic analyses

Additional specimens sequenced – ***Erysiphe magnifica*: JAPAN, Aichi Prefecture**, Nagoya-shi, Higashiyama Botanical Garden, on *Magnolia liliiflora*, 13 Oct. 2011, S. Takamatsu & J. Meeboon (MUMH5353; GenBank KJ567070, KJ567066). On *Magnolia* sp.: **ARGENTINA, Buenos Aires**, Japanese Garden, on *Magnolia* sp., 12 Mar. 2004, S. Takamatsu (MUMH2539; GenBank KJ567071).

Based on the molecular phylogenetic trees generated from the PhyML analyses, sequences generated from four isolates of *E. magnoliicola* (the Korean holotype, two other Korean isolates, and one Japanese isolate) formed an independent ITS clade with 99% bootstrap support, and an independent 28S clade with 87% bootstrap support (Figs 2–3). This lineage was separated from *E. magnoliae* and *E. magnifica* in ITS and 28S trees. Both phylogenetic analyses clearly support *E. magnoliicola* as an independent species.

Discussion

Erysiphe magnifica, described from the United States, has been recorded on *Magnolia* spp. throughout the world, including Korea and Japan (Braun & Cook 2012, Farr & Rossman 2014). It has also been found on lotus, *Nelumbo nucifera* Gaertn. (*Nelumbonaceae*), in a glasshouse in Germany and the identity of this unusual collection has been confirmed based on morphological characteristics and molecular sequence analysis (Kirschner 2010). *Erysiphe magnifica* differs from *E. magnoliicola* by its shorter conidiophore foot-cells (15–45 µm) and its more numerous chasmothecial appendages (5–23; Braun & Cook 2012). *Erysiphe magnoliae,* which has been reported on *M. obovata* in Korea and Japan, is clearly distinguished from *E. magnoliicola* by having conidiophores with shorter (15–25 µm) basally inflated foot-cells (Takamatsu et al. 2013). *Erysiphe bulbosa* is quite distinct from other species on *Magnolia* spp., and unique in having chasmothecial appendages with swollen bases (Braun 1988); the anamorph of *E. bulbosa* is still unknown.

Although *E. magnoliicola* is phylogenetically close to *E. magnoliae,* the sequence analyses (Figs 2–3) clearly distinguish two species and support the hypothesis that they have diverged from a common ancestor; *E. magnoliae* is sister to the *E. magnoliicola* clade with BS values of 89% (ITS) and 82% (28S). *Erysiphe magnifica,* which is not closely related to *E. magnoliae* and *E. magnoliicola*, may have developed independently from a separate lineage.

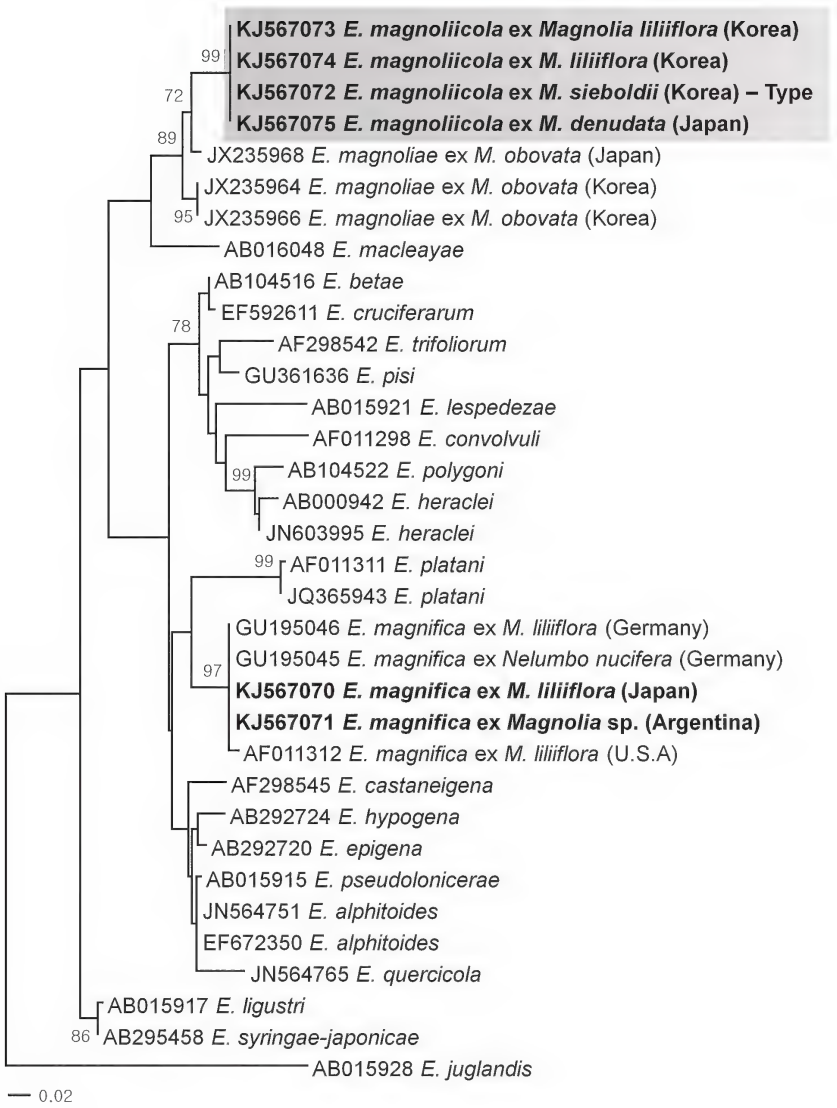


FIG. 2. Phylogenetic relationship between *Erysiphe* sequences and some reference isolates retrieved from GenBank, inferred by PhyML using the ITS rDNA region. Numbers above the branches represent the bootstrap values of over 70% obtained from 1000 bootstrap replicates. New sequences are indicated with bold font. Bar = number of nucleotide substitutions per site.

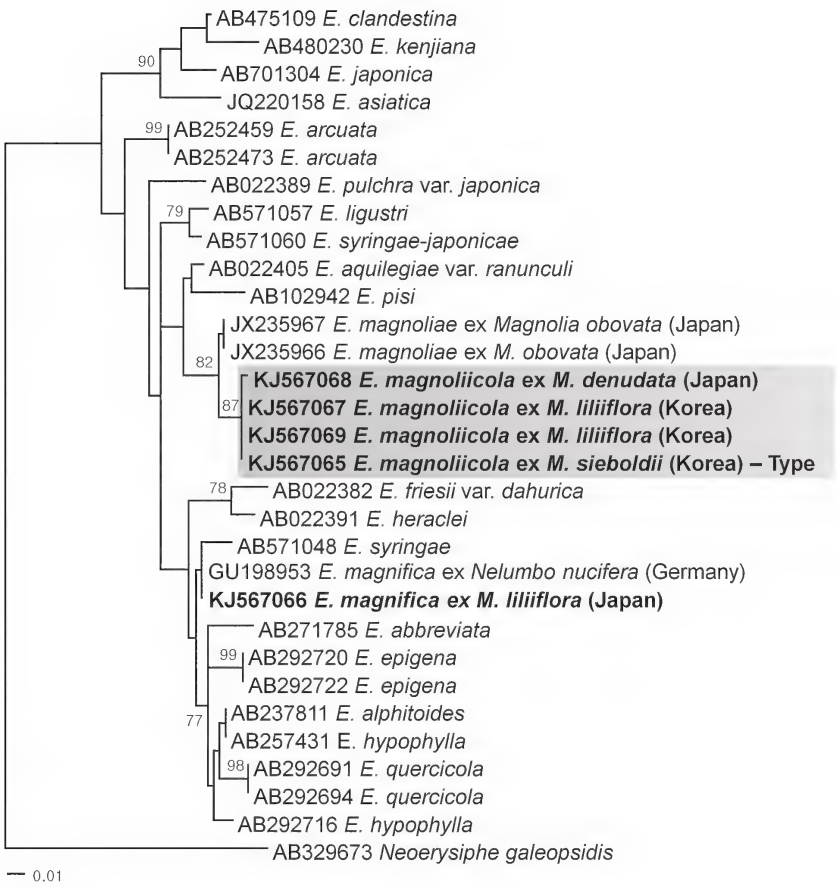


FIG. 3. Phylogenetic relationship between *Erysiphe* sequences and some reference isolates retrieved from GenBank, inferred by PhyML using the 28S rDNA region. Numbers above the branches represent the bootstrap values of over 70% obtained from 1000 bootstrap replicates. New sequences are indicated with bold font. Bar = number of nucleotide substitutions per site.

Molecular analyses of *E. bulbosa* have not yet been performed and are urgently required to address the phylogenetic relationships of powdery mildews on *Magnolia*.

Magnolia is a large genus, with about 210 species in *Magnoliaceae* subfam. *Magnolioideae,* including *M. liliiflora* and *M. denudata* native to China and *M. sieboldii* native to eastern Asia (China, Japan, and Korea; Azuma et al. 1999). These species are planted as ornamental trees throughout the world. Sequences

KJ567070 (Fig. 2) and KJ567066 (Fig. 3) came from a specimen of *E. magnifica* on *M. liliiflora* collected in the American Magnolia Garden in Higashiyama Botanical Garden, Nagoya, Japan. It has been suggested that the tree might have been imported from the United States and planted in the Japanese garden for ornamental purpose. ITS sequences retrieved from *E. magnifica* collections on *M. liliiflora* in North America, Argentina, and Germany and on *N. nucifera* in Germany have already been reported (Fig. 2). *Erysiphe magnifica* has been recorded on four *Magnolia* species (*M. kobus*, *M. obovata*, *M. liliiflora*, and *M. sieboldii)* in Korea (Shin 2000, Korean Society of Plant Pathology 2009). Although Lee (1975) recorded *E. magnifica* on *M. kobus,* no herbarium material has been preserved, and one of us (HDS) has not found any powdery mildews on *M. kobus* during his over 30 years' of field-work in Korea. Therefore, presence or identity of *E. magnifica* on *M. kobus* in Korea is obscure. Recently, powdery mildew caused by *E. magnoliae* (previously known as *M. magnifica* in Korea) on *M. obovata* in Korea and Japan was re-examined morphologically and phylogenetically (Takamatsu et al. 2013). We introduce *Erysiphe magnoliicola* as a new species on *M. liliiflora* and *M. sieboldii* in this study and confirm *E. magnoliae* and *E. magnoliicola* as causative agents of powdery mildews on *Magnolia* spp. in East Asia.

Key to species of *Erysiphe* sect. *Microsphaera* on *Magnolia* spp.

1. Chasmothecial appendages distinctly swollen at the base ..... ***E. bulbosa***
1. Chasmothecial appendages not swollen at the base **2**
2. Foot-cells of conidiophores inflated at the base ..... ***E. magnoliae***
2. Foot-cells of conidiophores not inflated at the base **3**
3. Foot-cells of conidiophores relatively short (15–40 μm); chasmothecia with 5–23 chasmothecial appendages ..... ***E. magnifica***
3. Foot-cells of conidiophores relatively long (42.5–70 μm); chasmothecia with 6–10 chasmothecial appendages ..... ***E. magnoliicola***

Acknowledgements

The authors thank Uwe Braun (Martin Luther University, Halle, Germany) and Wieslaw Mulenko (Maria Curie-Sklodowska University, Lublin, Poland) for serving as pre-submission reviewers. This work was supported by the BK21 Plus program in 2013 funded by National Research Foundation of Korea (NRF) and a grant from the Institute for Fermentation, Osaka, Japan.

Literature cited

Azuma H, Thien LB, Kawano S. 1999. Molecular phylogeny of *Magnolia* (*Magnoliaceae*) inferred from cpDNA sequences and evolutionary divergence of the floral scents. J. Plant Res. 112: 291–306. http://dx.doi.org/10.1007/PL00013885

Braun U. 1987. A monograph of the *Erysiphales* (powdery mildew). Beih. Nova Hedwigia 89: 1–700.

Braun U. 1988. *Microsphaera bulbosa* nom. nov. Mycotaxon 31: 171.

Braun U. 1995. The powdery mildews (*Erysiphales*) of Europe. G. Fischer, Jena.

Braun U, Cook RTA. 2012. Taxonomic manual of the *Erysiphales* (powdery mildews). CBS Biodiversity Series No.11. CBS, Utrecht.

Braun U, Takamatsu S. 2000. Phylogeny of *Erysiphe*, *Microsphaera*, *Uncinula* (Erysipheae) and *Cystotheca*, *Podosphaera*, *Sphaerotheca* (*Cystotheceae*) inferred from rDNA ITS sequences: some taxonomic consequences. Schlechtendalia 4: 1–33.

Braun U, Cook RTA, Inman AJ, Shin HD. 2002. The taxonomy of the powdery mildew fungi. 13–55, in: RR Bélanger et al. (eds). The powdery mildews: a comprehensive treatise. American Phytopathological Society Press, St. Paul, USA.

Darriba D, Taboada GL, Doallo R, Posada D. 2012. jModelTest 2: more models, new heuristics and parallel computing. Nat. Methods 9(8): 772. http://dx.doi.org/10.1038/nmeth.2109

Farr DF, Rossman AY. 2014. Fungal databases. Syst. Mycol. Microbiol. Lab., Online publication, ARS, USDA. Retrieved January 17, 2014.

Glawe DA. 2003. First report of powdery mildew of *Magnolia* caused by *Microsphaera magnifica* (*Erysiphe magnifica*) in the Pacific Northwest. Plant Health Progress (online). http://dx.doi.org/10.1094/PHP-2003-0512-02-HN

Glawe DA. 2008. The powdery mildews: a review of the world's most familiar (yet poorly known) plant pathogens. Annu. Rev. Phytopathol. 46: 27–51. http://dx.doi.org/10.1146/annurev.phyto.46.081407.104740

Guindon S, Gascuel O. 2003. A simple, fast and accurate algorithm to estimate large phylogenies by maximum likelihood. Syst. Biol. 52: 696–704. http://dx.doi.org/ 10.1080/10635150390235520

Hirata T, Takamatsu S. 1996. Nucleotide diversity of rDNA internal transcribed spacers extracted from conidia and cleistothecia of several powdery mildew fungi. Mycoscience 37: 283–288. http://dx.doi.org/10.1007/BF02461299

Katoh K, Standley DM. 2013. MAFFT multiple sequence alignment software version 7: improvements in performance and usability. Mol. Biol. Evol. 30(4): 772–780. http://dx.doi.org/10.1093/molbev/mst010

Kirschner R. 2010. First record of *Erysiphe magnifica* on lotus, a host outside the *Magnoliales.* Mycol. Prog. 9: 417–424. http://dx.doi.org/10.1007/s11557-009-0651-z

Korean Society of Plant Pathology. 2009. List of plant diseases in Korea. 5th ed. Suwon, Korea.

Lee HJ. 1975. Unrecorded causal organisms of Korean powdery mildews (IV). Bull. Hyosong Women's Coll. 16: 439–449.

Nomura Y. 1997. Taxonomical study of Erysiphaceae of Japan. Yokendo Ltd., Tokyo, Japan.

Shin HD. 1988. *Erysiphaceae* of Korea. Thesis, Department of Agricultural Biology, Graduate School of Seoul National University.

Shin HD. 2000. *Erysiphaceae* of Korea. National Institute of Agricultural Science and Technology, Suwon, Korea.

Takamatsu S, Heluta V, Havrylenko M, Divarangkoon R. 2009. Four powdery mildew species with catenate conidia infect *Galium*: molecular and morphological evidence. Mycol. Res. 113(1): 117–129. http://dx.doi.org/10.1016/j.mycres.2008.09.006

Takamatsu S, Cho SE, Meeboon J, Shin HD. 2013. Revisiting *Erysiphe magnoliae* with morphological and molecular data. Sydowia 65: 13–20.

Walsh PS, Metzger DA, Higuchi R. 1991. Chelex 100 as a medium for simple extraction of DNA for PCR-based typing from forensic material. BioTechniques 10: 506–513.

Wang AQ. 1987. A new genus of powdery mildew. Acta Mycologica Sinica 6: 74–76.

ISSN (print) 0093-4666 © 2014. Mycotaxon, Ltd. ISSN (online) 2154-8889

MYCOTAXON

http://dx.doi.org/10.5248/129.163

Volume 129(1), pp. 163–168 *July–September 2014*

Phoma recepii sp. nov. from the *Caloplaca cerina* group in Turkey

Mehmet Gökhan Halici[1*], Mehmet Candan[2], Mithat Güllü[1], & Ahmet Özcan[1]

[1] *University of Erciyes, Faculty of Science, Department of Biology 38039 Kayseri, Turkey*

[2] *University of Anadolu, Faculty of Science, Department of Biology Eskişehir, Turkey*

* Correspondence to: *mghalici@gmail.com*

Abstract — *Phoma recepii* is described from the apothecia of epiphytic *Caloplaca cerina* and *C. monacensis* in the western part of Turkey. The new species is characterized by having the narrowest conidia amongst the lichenicolous *Phoma* species. *Phoma caloplacae* is reported from Turkey for the first time.

Key words — biodiversity, coelomycetes, lichens, mitosporic fungi, *Teloschistaceae*

Introduction

Phoma Sacc. comprises approximately 220 species, most of which are plant pathogens, endophytes, or saprophytes (Boerema et al. 2004). Within this large genus, 23 species are obligately lichenicolous (Hawksworth 1981, Hawksworth & Cole 1994, Diederich et al. 2007, Brackel 2008, Kondratyuk et al. 2010, Lawrey et al. 2012). According to Lawrey et al. (2012), the multilocus phylogeny of *Phoma* places this genus in the *Phaeosphaeriaceae* (*Pleosporales*, *Dothideomycetes*).

Of the 182 species of lichenicolous fungi known from Turkey (Halıcı et al. 2012), only one *Phoma* species, *P. peltigerae* (P. Karst.) D. Hawksw. on *Peltigera* spp., has been reported from the country (Candan et al. 2010; Halıcı et al. 2012). During the Turkish *Teloschistales* project we discovered a new *Phoma* species on the apothecia of *Caloplaca cerina* and *C. monacensis*, and found *P. caloplacae* for the first time in Turkey.

Material & methods

Sections were prepared by hand and examined in I [Lugol's iodine (Merck 9261) with (KI) and without (I) pre-treatment with 10% KOH], 10% KOH, cotton blue, and water.

Conidia and pycnidia, which were measured in water, are given as: (min–)X–sd–*X*–X+sd(–max.), where 'min.' and 'max.' represent the extreme values, 'X' the arithmetic mean, and 'sd' the corresponding standard deviation. The microphotographs were taken with a Leica DFC 420 digital microscope camera with a c-mount interface and with a 5 megapixel CCD. The type of the new species is deposited in Erciyes University Herbarium Kayseri, Turkey (EUH).

Taxonomy

Phoma recepii Halıcı & Candan, **sp. nov.** FIGURE 1

MycoBank MB 810538

Differs from all lichenicolous *Phoma* species in having the narrowest conidia.

TYPE: Turkey, Denizli, south-east of Honaz Mount National Park, northwest of Tavas, 37°41′N 29°15′E, alt. 1750 m, *Juniperus* communities, calcareous motherrock, on apothecia of *Caloplaca monacensis* on *Juniperus* sp., 5 July 2012, M.G. Halıcı & M. Candan (**Holotype**, EUH (MGH 0.3441)).

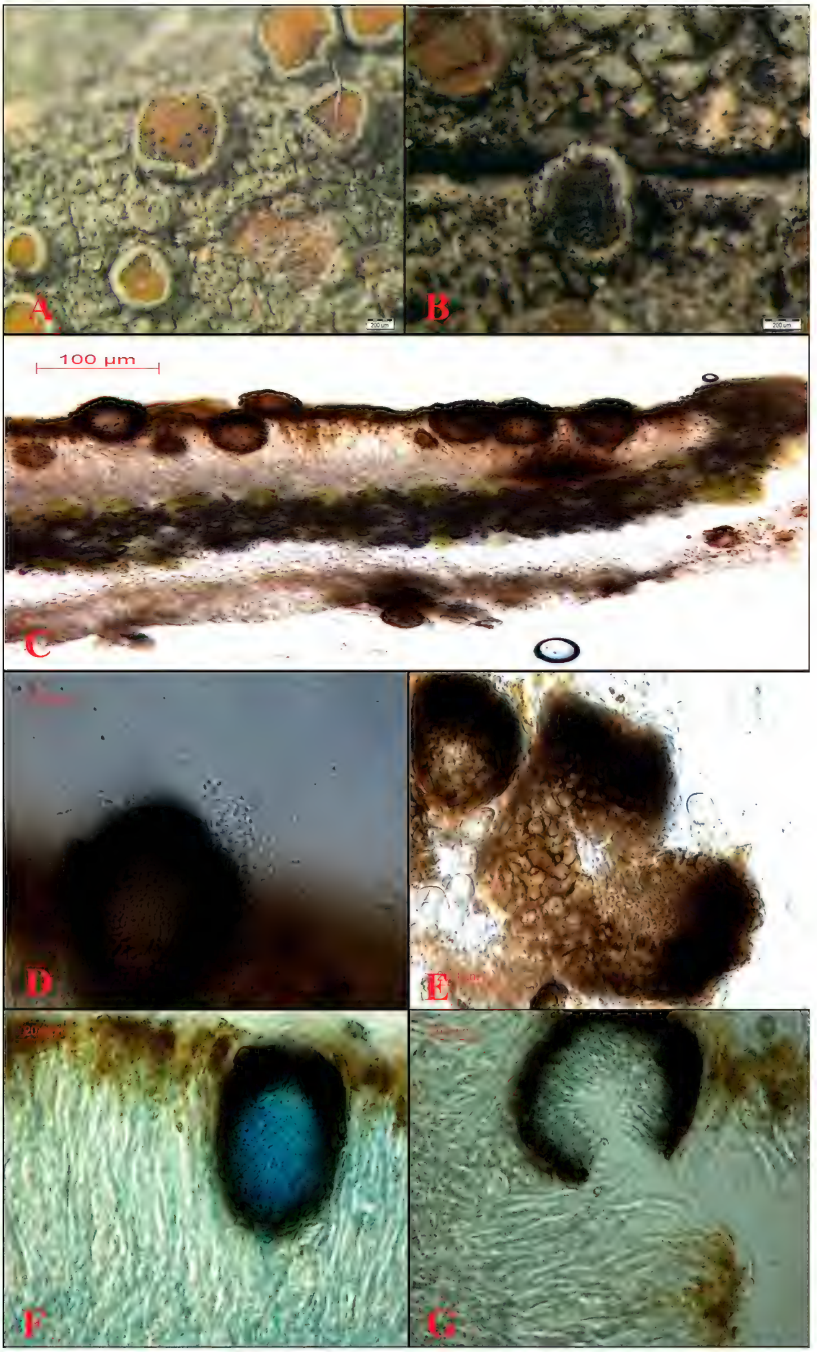
ETYMOLOGY: The epithet honors Recep Yazıcıoğlu, the former governor of Denizli Province, who was known as "Super Governor" by the public and was killed in a traffic accident in 2 September 2003.

Conidiomata pycnidial, first immersed in the apothecia of the host lichen (*Caloplaca cerina, C. monacensis*), but at maturity becoming partially erumpent, scattered, black in macroscopical view, in section brown in lower parts and dark brown in the upper parts, covered by a hyaline gelatinous sheath, subglobose to almost globose, (50–)54.5–*62*–69(–80) × (42–)46.5–*50*–55(–65) µm (n = 20), ostiolate, ostiole c. 10 µm diam; pycnidial wall 8–14 µm thick, composed of 2–3 layers of pseudoparenchymatous cells, mainly polyhedral but some globose, outer cells brown, about 4.5–7.5 × (3–)4–5(–6.5) µm, inner ones hyaline, 4–7 µm thick. Cells near the ostiole have a darker brownish tinge. Conidiogenous cells lining the inner wall of the pycnidial cavity, short ampulliform to subglobose, hyaline, smooth-walled, 6–9 × 3.5–5.5 µm, conidiogenesis enteroblastic. Conidia abundantly produced, arising singly, narrowly ellipsoid to almost bacilliform, rounded at the ends, hyaline, simple, smooth-walled, 2.5–*3*–3.5 × 1 µm (n = 100).

ADDITIONAL SPECIMEN EXAMINED: **TURKEY**, **MANISA**, Akhisar, south-west of Dağdere Village, 38°58′N 28°01′E, alt. 855 m, *Juniperus* communities, calcareous motherrock, on apothecia of *Caloplaca cerina* on *Juniperus* sp., 30 July 2012, M.G. Halıcı & M. Candan (MGH 0.3443).

ECOLOGY & DISTRIBUTION: *Phoma recepii* is currently known only from two localities in the inner parts of Aegean Region, Turkey on the apothecia

FIG. 1. ***Phoma recepii*** (Holotype, MGH 0.3441): A, B, Infected apothecial discs of the host lichen *Caloplaca monacensis*; C, F, Pycnidia immersed in the hymenium of the host lichen (F in cotton blue); D, Conidia; E, Surface view of pycnidial wall; G, Conidiogenous cells making a line inside the pycnidia.


A
B
100 µm
C
D
E
F
G

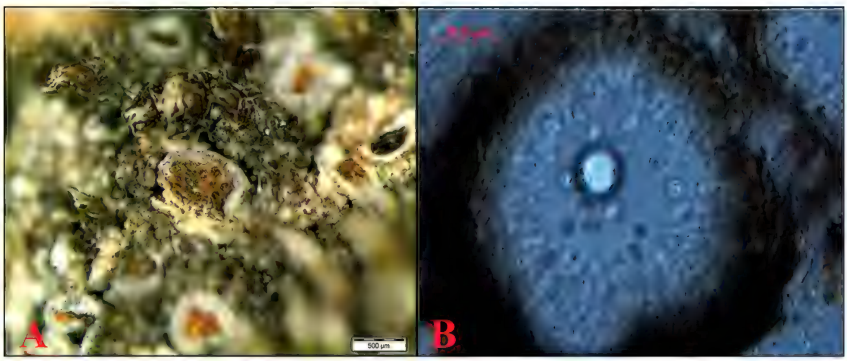


Fig. 2. ***Phoma caloplacae*** (MGH 0.7216): A, Infected apothecial disc of the host lichen *Caloplaca stillicidiorum*; B, Conidiogenous cells and conidia.

of *Caloplaca cerina* and *Caloplaca monacensis* on bark of *Juniperus* spp. at high elevations (850–1750 m). The species seems to be weakly pathogenic in the beginning, as the infected apothecial discs of the host lichen become discoloured and brownish. Also as seen in the section, ascospore production appears to be inhibited in infected parts of the hymenium. Finally it completely damages the infected apothecia of the host lichen. As the host lichen has a wide distribution in the northern hemisphere, *P. recepii* may have a similarly wide distribution.

Notes: Four *Phoma* species (*P. caloplacae*, *P. fuliginosa*, *P. pisutii*, *P. xanthomendozae*) have previously been described on the apothecia of members of the *Teloschistales,* all of which have longer and wider conidia than *P. recepii* (Table 1).

Similar to *P. recepii, P. caloplacae* D. Hawksw. grows in the hymenium of *Caloplaca cerina* but clearly differs from *P. recepii* in producing subglobose conidia (Hawksworth 1981). We also collected *P. caloplacae* in the central part of Anatolia on apothecia of *Caloplaca stillicidiorum* (Figure 2), which represents a new species record for Turkey:

> *Phoma caloplacae* specimens examined: **Turkey**, **Kayseri**, İncesu, Gökdağ, Western slopes of Mount Erciyes, 38°34′N 35°19′E, alt. 1750 m, crevices of siliceous rocks, on apothecia of *Caloplaca stillicidiorum* on mosses, 24 July 2003, M.G. Halıcı (MGH 0.7216); **Konya**, Seydişehir, Seydişehir-Beyşehir highway, NW of Nohuttaş Position, 37°29′N 31°49′E, alt. 1200 m, crevices of siliceous rocks, on apothecia of *Caloplaca stillicidiorum* on mosses, 14 June 2013, M.G. Halıcı & M. Candan (MGH 0.7217).

Phoma fuliginosa M.S. Cole & D. Hawksw., described on the apothecia of *Xanthomendoza trachyphylla* from USA (Nebraska), has larger and longer conidiogenous cells and conidia (Hawksworth & Cole 2004). *Phoma pisutii*

Table 1. Comparison of *Phoma* species growing on *Teloschistales* members

Species	Conidiomata (µm)	Conidiogenous cells (µm)	Conidia (µm)	Reference
P. caloplacae	50–130	5–6	4–7	Hawksworth (1981)
P. fuliginosa	50–75	5–7.5 × 3–6	5–6.5 × 3	Hawksworth & Cole (1994)
P. pisutii	110–175	3.5–4.5 × 2.5–3.5	4–7 × 2–3	Kondratyuk et al. (2010)
P. recepii	50–80 × 42–65	6–9 × 3.5–5.5	2.5–3–3.5 × 1	Present paper
P. xanthomendozae	140–160	5–10 × 2.5–3.5	4.5–8.5 × 3–4.5	Lawrey et al. (2012)

S.Y. Kondr. et al., described on *Xanthomendoza ulophyllodes* from Ukraine and USA, has much larger pycnidia and wider conidia (Kondratyuk et al. 2010). *Phoma xanthomendozae* Diederich & Freebury, recently described on *Xanthomendoza hasseana* and *X. montana* from Canada, has much larger pycnidia and wider conidia (Lawrey et al. 2012).

Phoma recepii has the narrowest conidia among the lichenicolous *Phoma* species. *Phoma lecanorina* Diederich, which has pycnidia immersed in the host thallus of *Lecanora expallens*, has longer and slightly wider conidia (3.2–5 × 1.2–1.6 µm) and smaller pycnidia (15–60 µm; Diederich 1986). *Phoma puncteliae* Diederich & Lawrey, described on *Punctelia rudecta* from USA, has smaller pycnidia (40–60 µm) and wider conidia ((2.3–)2.5–3(–3.1) × (1.9–)2–2.4(–2.5) µm; Lawrey et al. 2012). *Phoma everniae* D. Hawksw., a minute species known on older basal fronds of *Evernia prunastri* in UK, has smaller pycnidia ((20–)30(–35) µm in diam.) and longer and slightly wider conidia (4.5–5 × 1–1.5 µm; Hawksworth 1994). *Phoma dubia* (Linds.) Sacc. & Trotter, a species known on *Usnea* spp. in New Zealand, has longer and slightly wider conidia (3.5–5 × 1.5–2 µm) and possesses golden brown cells with thicker walls near the ostiole (Hawksworth 1981).

Acknowledgements

The manuscript was reviewed prior to submission by Kerry Knudsen (USA) and Wolfgang von Brackel (Germany). This study was financially supported by TÜBİTAK (111T927 coded project) and FDA-2013-4422 coded Erciyes University project.

Literature cited

Boerema GH, Gruyter J de, Noordeloos ME, Hamers MEC. 2004. *Phoma* identification manual. Differentiation of specific and infra-specific taxa in culture. CABI Publishing, Wallingford, UK.

Brackel W. 2008. *Phoma ficuzzae* sp. nov. and some other lichenicolous fungi from Sicily, Italy. Sauteria 15: 103–120.

Candan M, Halıcı MG, Özdemir Türk A. 2010. New records of peltigericolous fungi from Turkey. Mycotaxon 111: 149–153. http://dx.doi.org/10.5248/111.149

Diederich P. 1986. Lichenicolous fungi from the Grand Duchy of Luxembourg and surrounding areas. Lejeunia 119: 1–26.

Diederich P, Kocourková J, Etayo J, Zhurbenko M. 2007. The lichenicolous *Phoma* species (coelomycetes) on *Cladonia*. Lichenologist 39: 153–163.

Halıcı MG. 2008. A key to the lichenicolous *Ascomycota* (including mitosporic fungi) of Turkey. Mycotaxon 104: 253–286.

Halıcı MG, Candan M, Özdemir Türk A. 2012. A key to the peltigericolous fungi in Turkey. Mycotaxon 119: 277–289. http://dx.doi.org/10.5248/119.277

Hawksworth DL. 1981. The lichenicolous coelomycetes. Bulletin of the British Museum (Natural History), Botany 9: 1–98.

Hawksworth DL. 1994. Notes on British lichenicolous fungi: VII. Lichenologist 26: 337–347.

Hawksworth DL, Cole MS. 2004. *Phoma fuliginosa* sp. nov., from *Caloplaca trachyphylla* in Nebraska, with a key to the known lichenicolous species. Lichenologist 36: 7–13.

Kondratyuk S, Kärnefelt I, Goward T, Galloway D, Kudratov I, Lackovičová A, Lisická E, Guttová A. 2010. Diagnoses of new taxa. 435–445 in: AM Oksner (ed.). Flora lišaynikiv Ukraini y dvoch tomach, Tom 2, Vypusk 3. Kiiv, Naukovo Dumka.

Lawrey JD, Diederich P, Nelsen MP, Freebury C, Van den Broeck D, Sikaroodi M, Ertz D. 2012. Phylogenetic placement of lichenicolous *Phoma* species in the *Phaeosphaeriaceae* (*Pleosporales, Dothideomycetes*). Fungal Diversity 55: 195–213.

ISSN (print) 0093-4666 © 2014. Mycotaxon, Ltd. ISSN (online) 2154-8889

MYCOTAXON

http://dx.doi.org/10.5248/129.169

Volume 129(1), pp. 169–179 *July–September 2014*

Geastrum from the Atlantic Forest in northeast Brazil — new records for Brazil

Julieth de Oliveira Sousa[1]*, Bianca Denise Barbosa da Silva[2], & Iuri Goulart Baseia[3]

[1]*Programa de Pós Graduação em Sistemática e Evolução, Universidade Federal do Rio Grande do Norte, Campus Universitário, PO Box 59072-970, Natal, RN, Brazil*

[2]*Programa de Pós Graduação em Biologia de Fungos, Universidade Federal de Pernambuco, Depto. Micologia, Centro de Ciências Biológicas, Av. Nelson Chaves, PO Box 50670-420, Recife, PE, Brazil*

[3]*Departamento de Botânica, Ecologia e Zoologia, Universidade Federal do Rio Grande do Norte, Campus Universitário, PO Box 59072-970, Natal, RN, Brazil*

*Correspondence to: *julieth.oliveira.sousa@gmail.com*

Abstract — Expeditions to remnants of the Brazilian Atlantic Forest in the Northeast Region resulted in three noteworthy *Geastrum* species. *Geastrum morganii* and *G. quadrifidum* are reported for the first time from Brazil, while *G. albonigrum* is reported for the second time in South America. Detailed descriptions with taxonomic observations, photos of basidiomes in situ, and SEM photos are provided.

Key words — *Geastraceae*, *Geastrales*, gasteroid fungi, taxonomy, biodiversity, neotropics

Introduction

The Atlantic Forest domain that covers most of the east South American coast represents one of the most extensive rainforests on the continent (Tabarelli et al. 2005). This biome, a biodiversity hotspot with a wide range of fungal species, is considered the second most threatened domain on the planet (Brooks et al. 2006, Myers et al. 2000). Northeastern Brazil encompasses four of the five centers of endemism that occur in the Brazilian Atlantic Forest and includes the most degraded sectors of this domain, harboring dozens of endangered species (Tabarelli et al. 2006).

In Brazil, knowledge of the gasteroid fungi in the Atlantic Forest has increased over the last ten years so that currently more than 40 gasteroid species have been recorded for this biome in the Northeast Region (Baseia 2004, 2005;

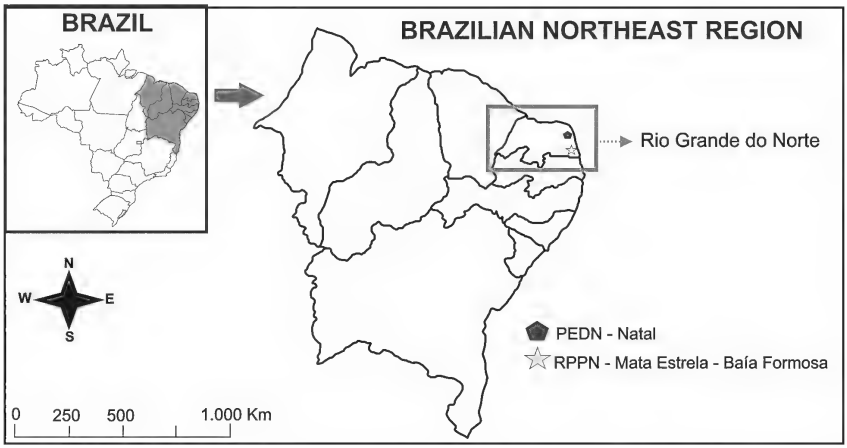


FIGURE 1. Map of Brazil's Northeast Region showing the remnants of Atlantic Rainforest studied.

Baseia et al. 2003a,b, 2006; Baseia & Calonge 2005, 2006, 2008; Leite & Baseia 2007; Leite et al. 2007a,b; Silva et al. 2007, 2013; Fazolino et al. 2008, 2010; Gurgel et al. 2008; Trierveiler-Pereira & Baseia 2009, 2011; Trierveiler-Pereira et al. 2009, 2010, 2011a; Barbosa et al. 2011; Cruz et al. 2012).

Sixteen *Geastrum* species have previously been reported from the northeastern Brazilian Atlantic Forest: *G. coronatum* Pers., *G. elegans* Vittad., *G. entomophilum* Fazolino et al., *G. fimbriatum* Fr., *G. fornicatum* (Huds.) Hook., *G. hieronymi* Henn., *G. hirsutum* Baseia & Calonge, *G. javanicum* Lév., *G. lageniforme* Vittad., *G. lloydianum* Rick, *G. ovalisporum* Calonge & Mor.-Arr., *G. saccatum* Fr., *G. schweinitzii* (Berk. & M.A. Curtis) Zeller, *G. setiferum* Baseia, *G. rusticum* Baseia et al., and *G. triplex* Jungh. These species were collected in various ecosystems including riparian, sand dunes, 'Restinga,' and coastal tableland. This work provides additional knowledge about *Geastrum* in Brazil.

Material & methods

Field expeditions were conducted during the rainy seasons of 2010 and 2012 to two Atlantic Forest remnants in Rio Grande do Norte State of northeastern Brazil (FIG.1): 'Parque Estadual Dunas de Natal' (PEDN) (5°50′31.23″S 35°11′39.21″W) and the 'Reserva Particular do Patrimônio Natural Mata Estrela' (RPPN Mata Estrela) (6°22′27.56″S 35°1′24.87″W). Tissues containing basidiospores, eucapillitium, and exoperidial hyphae were mounted in 5% KOH and then examined with an Olympus BX41 optical microscope, and scanning electron microscopy (SEM) was used to observe in detail basidiospore ornamentation, the eucapillitium, and the endoperidial surface

with a Phillips XL30-ESEM electronic microscope; preparation for SEM observation followed Silva et al. (2011). All measurements include basidiospore ornamentation. Basidiospore abbreviations follow Bates (2004): n = number of randomly measured basidiospores; x = mean ± standard deviation of basidiospore diameter and height (including ornamentation); Q_m = mean height/width quotient. Color descriptions were based on Kornerup & Wanscher (1978). All collected specimens were deposited in the Herbarium of the Federal University of Rio Grande do Norte, Natal, Brazil (UFRN).

Taxonomy

Geastrum albonigrum Calonge & M. Mata, Bol. Soc. Micol. Madrid 28: 332 (2004).

Plate 1

Unexpanded basidiome epigeous, globose to subglobose, 10–16 × 9–13 mm, surface hairy, brown (6E4) to dark brown (6E4), not encrusted with debris; rhizomorph ≤30 mm long, persistent. Expanded basidiome saccate, 10–13 × 17–26 mm. Exoperidium splitting into 5–7 revolute rays, non-hygroscopic. Mycelial layer grayish brown (6D3) to dark brown (6F4), hairy, not encrusted, peeling away in irregular patches with age exposing the fibrous layer. Fibrous layer white (4A1) to yellowish white (4A2), coriaceous, thick, glabrous, with rhizomorph attached. Pseudoparenchymatous layer dark brown (6F4, 6F3), glabrous to rimose, peeling away in irregular patches. Endoperidial body subglobose 11–13 × 10–11.5 mm, sessile, glabrous, dark brown (7F3), lightly pruinose, apophysis absent. Peristome fibrillose becoming lacerated with age, not delimited, concolorous with the endoperidium. Gleba grayish brown (7F2). Basidiospores globose to subglobose, 3.8–5 × 3.8–5 µm [x = 4.6 ± 0.2 × 4.6 ± 0.4 µm, Q_m = 1.01, n = 30], brownish, verrucose; warts short cylindrical with rounded tips; apiculus inconspicuous. Eucapillitium 2.5–5 µm diam., walls straight, encrusted, lumen present, surface verrucose, brown. Mycelial layer composed of sinuous-walled hyphae, 2.5–5 µm diam., lumen absent, brownish. Fibrous layer composed of straight-walled hyphae, 2.5–5 µm diam., greenish yellow to hyaline, lumen absent. Pseudoparenchymatous layer composed of thin-walled (<1 µm) hyphal cells, subglobose to pyriform hyphae, 26.6–53.3 × 16.5–34.2 µm, hyaline to yellowish.

Substrate: Sandy soil covered with litter and decaying wood.

Specimens examined: **BRAZIL. Rio Grande do Norte**: Baía Formosa, RPPN Mata Estrela, 14.VII.2011, leg. J.O. Sousa & B.D.B. Silva (UFRN-Fungos 1745); 14.VII.2011, leg. B.D.B. Silva & J.O Sousa (UFRN-Fungos 1747).

Distribution: Central America: Costa Rica (Calonge & Mata 2004); North America: Mexico (Calonge & Mata 2004); South America: Brazil, Mato Grosso State (Treirveiler-Pereira et al. 2011b), Rio Grande do Norte State (this paper).

Taxonomic remarks: *Geastrum albonigrum* is characterized by the pseudoparenchymatous layer and black endoperidium, white fibrous layer

Plate 1. *Geastrum albonigrum*. 1. Fresh basidiomes (UFRN-Fungos 1745). 2. Basidiome showing the rhizomorph (UFRN-Fungos 1747). 3. Eucapillitium (SEM) (UFRN-Fungos 1745). 4. Basidiospores (SEM) (UFRN-Fungos 1745).

with adhering rhizomorphs, and ephemeral hairy mycelial layer. *Geastrum coronatum*, *G. lloydianum*, and *G. ovalisporum* also exhibit dark endoperidia, but these species differ by arched basidiomes, pedicellate endoperidia with apophysis, and delimited peristomes (Ponce de Leon 1968, Sunhede 1989, Calonge et al. 2000, Bates 2004). *Geastrum hirsutum* has immature basidiomes very similar to those of *G. albonigrum*, being globose to subglobose with their surfaces covered with hairs. However, *G. hirsutum* exhibits distinctly expanded basidiomes with smaller basidiospores (2.5–3 µm), persistent mycelial layer, a delimited peristome, grayish endoperidium and the presence of a well-developed subiculum (Calonge & Mata 2004, Baseia & Calonge 2006). *Geastrum fimbriatum* is also a closely related species, distinguished by the light brown to grayish brown endoperidium, hairless mycelial layer with incrustations, and rhizomorphs that are absent in the expanded basidiome (Sunhede 1989, Bates 2004, Calonge & Mata 2004). Our SEM image of the eucapillitium demonstrating a verrucose surface and photos of the fresh expanded basidiome augment the protologue of *G. albonigrum*. This constitutes a second report for South America and a first report for northeastern Brazil for *Geastrum albonigrum*.

Plate 2. *Geastrum morganii*. 5–6. Fresh basidiome (UFRN- Fungos 1793) – (UFRN- Fungos 1744). 7. Basidiospores (SEM) (UFRN- Fungos 1793). 8. Surface of the endoperidium (SEM) (UFRN- Fungos 1793).

Geastrum morganii Lloyd, Myc. Writ. 1: 80 (1901). Plate 2

Unexpanded basidiome epigeous, lageniform, subglobose to irregular, 24 × 16 mm, surface papery to rugulose, brown (6E4) to yellowish brown (5D5), not encrusted, rhizomorph not observed. Expanded basidiome saccate to arched, 9–28 × 10–62 mm. Exoperidium splitting into 6–7 rays, revolute to arched, elongated with slender tips, non-hygroscopic. Mycelial layer brown (6E5) to dark brown (6F5), papery, not encrusted, persistent or peeling away with age. Fibrous layer yellowish white (4A2) to white orange (5A2), coriaceous, rigid. Pseudoparenchymatous layer dark brown (7F4), thick (≤3 mm when fresh), peeling away in irregular patches from the base of the rays, forming longitudinal cracks or a collar-like structure surrounding the endoperidium. Endoperidial body globose to subglobose, 6–24 × 7–26 mm, sessile, with protruding hyphae, orange brown (5C3) to light brown (6D4), apophysis absent. Peristome irregularly plicate, not delimited, conical, with 5–11 irregular folds, 2–4 mm high, lighter or darker than the endoperidium. Gleba dark brown (7F3). Basidiospores globose to subglobose, 3.8–6(–6.9) × 3.8–6(–6.8) µm [x = 5.3 ± 0.4 × 5.2 ± 0.4 µm, Q_m = 1.03, n = 30], dark brown, strongly verrucose; verrucae

long and columnar with plane tips, slightly truncated; apiculus inconspicuous. Eucapillitium 2.5–6 µm diam., walls sinuous thin (<1 µm), surface encrusted, verrucose, yellowish white. Mycelial layer composed of thick (>1 µm) sinuous-walled hyphae, 2.5–4.0 µm diam., lumen absent, clamp connections present, yellowish. Fibrous layer composed of straight-walled hyphae, 2.5–5 µm diam., lumen absent, hyaline to slightly greenish. Pseudoparenchymatous layer composed of thin-walled (<1 µm) hyphal cells, oval, ellipsoid to pyriform, 29.2–82.5 × 20–42 µm, hyaline to slightly brownish. Endoperidium with protruding hyphae agglutinated.

Substrate: Sandy soil covered with litter.

Specimens examined: **BRAZIL. Rio Grande do Norte:** Baía Formosa, RPPN Mata Estrela, 14.VII.2010, leg. B.D.B. Silva, J.O. Sousa & I.G. Baseia (UFRN-Fungos 1744); 14.VII.2011, leg. J.O. Sousa & B.D.B Silva (UFRN-Fungos 1746); 14.VII.2012, leg. J.O. Sousa, B.D.B. Silva & J.C. Bezerra (UFRN-Fungos 1793); Serra Negra do Norte, Estação Ecológica do Seridó, 19.IV.2008, leg. E.P Fazolino, B.B.B. Silva & J.J.S. Oliveira (UFRN-Fungos 816).

Distribution: Africa: Congo (Dissing & Lange 1962); Central America: Costa Rica (Calonge & Mata 2006); Europe: France (Sunhede 1989); North America: United States (Coker & Couch 1928), Hawaii (Hemmes & Desjardin 2011); South America: Argentina (Caffot et al. 2013), Brazil (this paper).

Taxonomic remarks: This species is characterized by its non-delimited, irregularly plicate peristome, a mycelial layer lacking incrustations, saccate to arched basidiomes, and a sessile endoperidium (Hemmes & Desjardin 2011, Sunhede 1989). Some *G. morganii* basidiomes may develop a collar-like structure around the endoperidium as in *G. triplex*, but the latter is distinguished by the delimited fibrillose peristome and basidiomes larger than 25–150 mm (Sunhede 1989). *Geastrum morganii* is also very similar to *G. elegans*, which differs by its distinctly delimited peristome with a larger number of regular folds (10–20), a mycelial layer encrusted with debris, and a slightly pruinose endoperidium (Sunhede 1989, Bates 2004, Calonge & Mata 2006). *Geastrum reticulatum* Desjardin & Hemmes from Hawaii is morphologically similar but differs from *G. morganii* in the immature basidiomata with hyphae projecting above the surface, as well as a regularly plicate peristome that is distinctly delimited and depressed in the endoperidium (Hemmes & Desjardin 2011). *Geastrum episcopale* F. Kuhar & L. Papin., which can be confused with *G. morganii*, has smaller basidiospores (approximately 2.2 µm in diam.), calcium oxalate crystals on the endoperidium and a magenta exoperidium and purple endoperidium (Kuhar & Papinutti 2009). Calonge (1998) considers *G. morganii* as the American form of *G. badium* Pers., but Sunhede (1989) regards them as different species. This is the first report of *G. morganii* from Brazil.

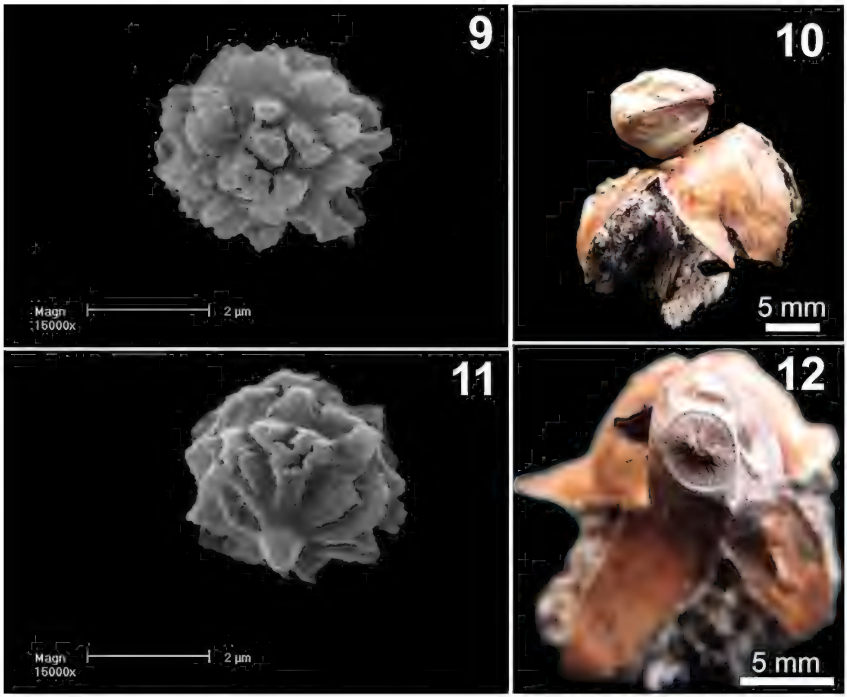


Plate 3. *Geastrum quadrifidum* (UFRN-Fungos 1748). 9–11. Basidiospores (SEM). 10–12. Dry basidiomes.

Geastrum quadrifidum Pers., Neues Mag. für die Bot. 1: 86 (1794). Plate 3

Unexpanded basidiome not observed. Expanded basidiome fornicate, 11 × 26 mm. Exoperidium splitting into 5 rays, fornicate, adhering to the mycelial layer, non-hygroscopic. Mycelial layer yellowish white (4A2), felted, encrusted with organic matter and sand, persistent, forming a cup under the basidiome. Fibrous layer white orange (5A2), papery, thin. Pseudoparenchymatous layer brown (6E5), rigid, peeling away in irregular patches with age to expose the fibrous layer. Endoperidial body subglobose, 8 × 9 mm, stalked, brownish orange (5C3), slightly pruinose. Stalk 1.5 mm high, distinct, lighter than the endoperidium. Apophysis reduced, concolorous with the endoperidium. Peristome fibrillose, distinctly delimited, delimitation white orange (5A2), mammiform, concolorous with to darker than endoperidium. Gleba dark brown (6F4). Basidiospores globose to subglobose, 3.8–6.3 µm × 3.8–6 µm [$x = 5.4 \pm 0.5 \times 5 \pm 0.7$, Qm = 1.1, n = 30], brownish, densely verrucose; verrucae long, columnar, truncate, with planar or confluent tips; apiculus prominent,

surrounded by columnar processes. Eucapillitium 1.9–6.3 µm diam., thick sinuous walls (>1 µm), encrusted, lumen absent, surface glabrous, yellowish brown. Mycelial layer composed of thin hyphae, <1 µm diam., hyaline. Fibrous layer composed of thin straight-walled (<1 µm) hyphae, 2.5–6.8 µm diam., lumen absent, hyaline to slightly yellowish. Pseudoparenchymatous layer composed of collapsed thick-walled (>1 µm) hyphal cells, subglobose to oval, brownish.

Substrate: Sandy soil.

Specimen examined: **BRAZIL. Rio Grande do Norte**: Natal, Parque Estadual Dunas do Natal, 3.IV.2012, leg. J.O. Sousa, R.H.S.F. Cruz & J.C Bezerra (UFRN-Fungos 1748).

Distribution: Africa: South Africa (Bottomley 1948); Asia: Japan (Kasuya et al. 2011); Europe: Spain (Calonge 1998), England (Pegler et al. 1995, Demoulin & Marriott 1981), Norway (Eckblad 1995), Estonian, Finland, Latvia, Sweden, Norway, (Sunhede 1989); North America: United States (Bates 2004), Hawaii: (Smith & Ponce de Leon 1982); South America: Argentina (Soto & Wright 2000), Brazil (this paper).

Taxonomic remarks: *Geastrum quadrifidum* is characterized by the fornicate basidiome, a small (4–6) number of rays, a mycelial layer forming a cup on the expanded basidiome, and a distinctly delimited fibrillose peristome (Bates 2004, Sunhede 1989). *Geastrum minimum* Schwein. is distinguished by larger (5–11) number of rays, a densely pruinose endoperidium, and basidiomes that are frequently not fornicate (Bates 2004, Sunhede 1989). *Geastrum quadrifidum* also resembles *G. fornicatum*, which differs by its larger basidiomes (≤40 mm wide), a non-delimited peristome, basidiospores with rarely confluent warts, and an endoperidium with projecting hyphae (Bates 2004, Sunhede 1989). Another closely related species, *G. leptospermum* G.F. Atk. & Coker, is distinguished by the smaller basidiomes (2–3 mm wide) (Bates 2004). The also similar *G. welwitschii* Mont. is distinguished by its indistinctly delimited peristome and a mycelial layer lacking incrustations (Calonge 1998). This is the first report of *G. quadrifidum* from Brazil.

Acknowledgments

The authors gratefully acknowledge the CTPETRO-INFRA and FINEP/LIEM for their collaboration with scanning electron microscopy. The authors express their thanks to Johannes C. Coetzee (Cape Peninsula University of Technology, Bellville, South Africa) and Felipe Wartchow (Universidade Federal da Paraíba, Brazil) for reviewing the manuscript. This work was financed by the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) and Conselho Nacional de Desenvolvimento Cientifico e Tecnológico (CNPq).

Literature cited

Barbosa MMB, Silva, MP, Cruz, RHSF, Calonge, FD, Baseia IG. First report of *Morganella compacta* (*Agaricales, Lycoperdaceae*) from South America. Mycotaxon 116: 381–386. http://dx.doi.org/10.5248/116.381

Baseia IG. 2004. Contribution to the study of the genus *Calvatia* (*Lycoperdaceae*) in Brazil. Mycotaxon 88: 107–112.

Baseia IG. 2005. Some notes on the genera *Bovista* and *Lycoperdon* (*Lycoperdaceae*) in Brazil. Mycotaxon 91: 81–86.

Baseia IG, Calonge FD. 2005. *Aseroë floriformis*, a new phalloid with a sunflower-shaped receptacle. Mycotaxon 91: 169–172.

Baseia IG, Calonge FD. 2006. *Geastrum hirsutum*: a new earthstar fungus with a hairy exoperidium. Mycotaxon 95: 301–304.

Baseia IG, Calonge FD. 2008. *Calvatia sculpta*, a striking puffball occurring on Brazilian sand dunes. Mycotaxon 106: 269–272.

Baseia IG, Cavalcanti MA, Milanez, AI. 2003a. Additions to our knowledge of the genus *Geastrum* (*Phallales: Geastraceae*) in Brazil. Mycotaxon 85: 409–416.

Baseia IG, Gibertoni TB, Maia, LC. 2003b. *Phallus pygmaeus*, a minute species from a Brazilian tropical rain forest. Mycotaxon 85: 77–80.

Baseia IG, Calonge FD, Maia LC. 2006. Notes on the *Phallales* in Neotropics. Boletín de la Sociedad Micológica de Madrid 30: 87–93.

Bates ST. 2004. Arizona members of the *Geastraceae* and *Lycoperdaceae* (*Basidiomycota*, *Fungi*). Master's Thesis. Arizona State University, Tempe.

Bottomley AM. 1948. Gasteromycetes of South Africa. Bothalia 4: 473–810.

Brooks TM, Mittermeier RA, Fonseca GAB, Gerlach J, Hoffmann M, Lamoreux JF, Mittermeier CG, Pilgrim JD, Rodrigues ASL. 2006. Global biodiversity conservation priorities. Science 13: 58–61. http://dx.doi.org/10.1126/science.1127609

Cabral TS, Silva BDB, Marinho P, Baseia IG. 2014. *Geastrum rusticum* (Geastraceae, Basidiomycota), a new earthstar fungus in the Brazilian Atlantic rainforest – a molecular analysis. Nova Hedwigia 98: 265–272. http://dx.doi.org/10.1127/0029-5035/2013/0158

Caffot MLH, Robledo G, Domínguez LS. 2013. Gasteroid mycobiota (*Basidiomycota*) from *Polylepis australis* woodlands of central Argentina. Mycotaxon 123-491. http://dx.doi.org/10.5248/123.491

Calonge FD. 1998. *Gasteromycetes*. I. *Lycoperdales*, *Nidulariales*, *Phallales*, *Sclerodermatales*, *Tulostomatales*. Flora Mycologica Iberica 3: 1–271.

Calonge FD, Mata M. 2004. A new species of *Geastrum* from Costa Rica and Mexico. Boletín de la Sociedad Micológica de Madrid 28: 331–335.

Calonge FD, Mata M. 2006. Adiciones e Correcciones al Catálago de *Gasteromycetes* da Costa Rica. Boletín de la Sociedad Micológica de Madrid 30: 111–119.

Calonge FD, Moreno-Arroyo B, Gómez J. 2000. Aportación al conocimiento de los *Gasteromycetes*, *Basidiomycotina*, de Bolivia (América del Sul). *Geastrum ovalisporum* sp. nov. Boletín de la Sociedad Micológica de Madrid 25: 271–275.

Coker WC, Couch JN. 1928. The *Gasteromycetes* of the eastern United States and Canada. University of North Carolina Press, Chapel Hill, 283 p.

Cruz RHSF, Barbosa M, Baseia IG. 2012. *Cyathus badius* and *C. earlei* reported from Brazilian Atlantic rain forest. Mycotaxon 121: 365–369. http://dx.doi.org/10.5248/121.365

Demoulin V, Marriott JVR. 1981. Key to the *Gasteromycetes* of Great Britain. Bulletin of the British Mycological Society 15: 37–56.

Dissing H, Lange M. 1962. *Gasteromycetes* of Congo. Bulletin du Jardin Botanique de l'État 32: 325–416.

Eckblad FE. 1955. The *Gasteromycetes* of Norway. The epigean genera. Nytt Magasin for Botanikk 4: 19–86.

Fazolino EP, Calonge FD, Baseia IG. 2008. *Geastrum entomophilum*, a new earthstar with an unusual spore dispersal strategy. Mycotaxon 104: 449–453.

Fazolino EP, Trierveiler-Pereira L, Calonge FD, Baseia IG. 2010. First records of *Clathrus* (*Phallaceae*, *Agaricomycetes*) from the Northeast Region of Brazil. Mycotaxon 113: 195–202. http://dx.doi.org/10.5248/113.195

Gurgel FE, Silva BDB, Baseia IG. 2008. New records of *Scleroderma* from Northeastern Brazil. Mycotaxon 105: 399–405.

Hemmes DE, Desjardin DE. 2011. Earthstars (*Geastrum, Myriostoma*) of the Hawaiian Islands including two new species, *Geastrum litchiforme* and *Geastrum reticulatum*. Pacific Science 65: 477–496. http://dx.doi.org/10.2984/65.4.477

Kasuya T, Hosaka K, Sakamoto H, Uchida A, Hoshino T, Kakishima M. 2011. New records of *Geastrum* from Japanese sand dunes. Mycotaxon 118: 1–15. http://dx.doi.org/10.5248/118.1

Kornerup A, Wansher JE. 1978. Methuen handbook of colour, 3rd edn. Eyre Methuen, London. 252 p.

Kuhar F, Papinutti L. 2009. *Geastrum episcopale*: a new noticeable species with red-violet exoperidium. Mycologia 101: 535–538. http://dx.doi.org/10.3852/08-029

Leite AG, Baseia IG. 2007. Novos registros de *Geastraceae* Corda para o Nordeste Brasileiro. Sitientibus. Série Ciências Biológicas 7: 178–183.

Leite AG, Calonge FD, Baseia IG. 2007a. Additional studies on *Geastrum* from Northeastern Brazil. Mycotaxon 101: 103–111.

Leite AG, Silva BDB, Araújo RS, Baseia IG. 2007b. Espécies raras de *Phallales* (*Agaricomycetidae, Basidiomycetes*) no Nordeste do Brasil. Acta Botanica Brasilica 21: 119–124.

Myers N, Mittermeier RA, Mittermeier CG, Fonseca GAB, Kent J. 2000. Biodiversity hotspots for conservation priorities. Nature 403: 853–858. http://dx.doi.org /10.1038/35002501

Pegler DN, Læssøe T, Spooner BM. 1995. British puffballs, earthstars, and stinkhorns. Royal Botanic Gardens, Kew. 211 p.

Ponce de León P. 1968. A revision of the family *Geastraceae*. Fieldiana Botany 31: 302–349.

Silva BDB, Calonge FD, Baseia IG. 2007. Studies on *Tulostoma* (*Gasteromycetes*) in the Neotropics. Some Brazilian species. Mycotaxon 101: 47–54.

Silva BDB, Sousa JO, Baseia IG. 2011. Discovery of *Geastrum xerophilum* from the Neotropics. Mycotaxon 118: 355–359. http://dx.doi.org/10.5248/118.355

Silva BDB, Cabral TS, Marinho P, Ishikawa NK, Baseia IG. 2013. Two new species of *Geastrum* (*Geastraceae*, *Basidiomycota*) from Brazil. Nova Hedwigia 96: 445–456.

Smith CW, Ponce de Leon P. 1982. Hawaiian gasteroid fungi. Mycologia 74: 712–717.

Soto MK, Wright JE. 2000. Taxonomia del genero *Geastrum* (*Basidiomycetes, Lycoperdales*) em la Provincia de Buenos Aires, Argentina. Boletín de la Sociedad Argentina de Botanica 34: 185–201.

Sunhede S. 1989. *Geastraceae* (*Basidiomycotina*). Morphology, ecology, and systematics with special emphasis on the North European species. Synopsis Fungorum 1. 534 p.

Tabarelli M, Pinto LP, Silva JMC, Hirota M, Bedê L. 2005. Challenges and opportunities for biodiversity conservation in the Brazilian Atlantic Forest. Conservation Biology 19: 695–700.

Tabarelli M, Melo MDVC, Lira OC. 2006. Nordeste e Estados do Nordeste, menos Sergipe. 149–164. In: M. Campanili& M. Prochnow (eds.). Mata Atlântica: Uma rede pela floresta. Brasília, Rede de ONGs da Mata Atlântica (RMA).

Trierveiler-Pereira L, Baseia IG. 2009. Revision of the Herbarium URM IV. *Nidulariaceae* (*Basidiomycota*). Nova Hedwigia 89: 361–369.

Trierveiler-Pereira L, Baseia IG. 2011. Contribution to the knowledge of gasteroid fungi (*Agaricomycetes*, *Basidiomycota*) from the state of Paraíba, Brazil. Revista Brasileira de Biociências 9: 167–173.

Trierveiler-Pereira L, Bezerra KMT, Bezerra JL, Baseia IG. 2009. First records of *Geastraceae* and *Nidulariaceae* (*Basidiomycota*, *Fungi*) from Bahia, Northeastern Brazil. Biociências (Porto Alegre), 7: 316–319.

Trierveiler-Pereira L, Kreisel H, Baseia IG. 2010. New data on puffballs (*Agaricomycetes*, *Basidiomycota*) from the Northeast Region of Brasil. Mycotaxon 111: 411–421. http://dx.doi.org/10.5248/111.411

Trierveiler-Pereira L, Calonge FD, Baseia IG. 2011a. New distributional data on *Geastrum* (*Geastraceae, Basidiomycota*) from Brazil. Acta Botanica Brasilica 25: 577–585.

Trierveiler-Pereira L, Gomes–Silva AC, Baseia IG. 2011b. Observations on gasteroid *Agaricomycetes* from the Brazilian Amazon rainforest. Mycotaxon 118: 273–282. http://dx.doi.org/10.5248/118.273

ISSN (print) 0093-4666 © 2014. Mycotaxon, Ltd. ISSN (online) 2154-8889

MYCOTAXON

http://dx.doi.org/10.5248/129.181

Volume 129(1), pp. 181–186 *July–September 2014*

Ypsilomyces, a new thallic genus of conidial fungi from the semi-arid Caatinga biome of Brazil

Davi Augusto Carneiro de Almeida* &
Luís Fernando Pascholati Gusmão

Universidade Estadual de Feira de Santana,
Av. Transnordestina, S/N – Novo Horizonte, 44036-900. Feira de Santana, BA, Brazil

* Correspondence to: *daviaugusto@gmail.com*

Abstract — During research on conidial fungi from the semi-arid region of Brazil, a unique specimen was collected on decaying leaves. *Ypsilomyces elegans* gen. et sp. nov. is characterized by macronematous mononematous conidiophores occurring on pseudoparenchymatous tissue, terminal conidiogenous cells extending percurrently, and thallic septate Y-shaped subhyaline conidia. Descriptions, illustrations, and comments are provided.

Key words — biodiversity, hyphomycetes, tropical microfungi

Introduction

Studies conducted in the semi-arid region of Brazil in the beginning of this century have revealed several novel taxa and new records of conidial fungi (Barbosa & Gusmão 2005, Barbosa et al. 2007, 2013, Cruz et al. 2007, 2012, Gusmão et al. 2008, Almeida et al. 2011, 2012, 2013, Santa Izabel et al. 2013). The Program of Research on Biodiversity in the Brazilian semi-arid (PPBIO) has contributed to the knowledge on biodiversity of fungi in Brazil by funding the majority of the aforementioned researches. Although several new taxa of fungi have been reported, this region continues to be a rich source of novel taxa.

During a survey of conidial fungi on plant debris in an area of extreme biological importance in the Brazilian semi-arid region (Velloso et al. 2002), an interesting specimen was found. Since its morphological features did not match any known genera of conidial fungi, a new genus is established herein.

Materials & methods

Samples of decaying leaves were collected at Serra da Fumaça, Pindobaçu, Bahia state, during August–December 2008. Description of the study area and methods for

specimen collection, isolation, and morphological examination are provided in Almeida et al. (2011). Fungal structures were measured using a BX51 Olympus microscope equipped with bright field and Nomarski interference optics; images were photographed with a DP25 Olympus digital camera and processed using Adobe Photoshop CS6 and Image J. Drawings were made directly from digital images using the technique described by Barber & Keane (2007). The holotype specimen was deposited in the Herbarium of Universidade Estadual de Feira de Santana (HUEFS). An attempt was made to cultivate the fungus but unfortunately we did not have success.

Taxonomy

Ypsilomyces D.A.C. Almeida & Gusmão, **gen. nov.** FIGS. 1, 2

MYCOBANK MB 807123

Differs from *Biflagellospora*, *Diademospora*, *Diplocladiella*, *Iyengarina*, *Vanterpoolia*, and *Weufia* by thallic conidiogenesis.

TYPE SPECIES: *Ypsilomyces elegans* D.A.C. Almeida & Gusmão

ETYMOLOGY: from the Latin, *ypsilon*, referring to the Y-shaped conidia and the Greek, *myces*, referring to fungus.

CONIDIOPHORES macronematous, mononematous, unbranched or branched, cylindrical, erect, straight or flexuous, septate, smooth, pale brown to brown. STROMATA pseudoparenchymatous, brown to dark brown. CONIDIOGENOUS cells terminal, cylindrical, smooth, pale brown, with enteroblastic percurrent extension that produce fertile branches, which forming thallic-arthric conidia after disarticulation. CONIDIA thallic-arthric, single, dry, smooth, septate, usually more or less dichotomous branched, Y-shaped, furcated or cylindrical, subhyaline, produced after disarticulation of the fertile branches.

Ypsilomyces elegans D.A.C. Almeida & Gusmão, **sp. nov.** FIGS 1, 2

MYCOBANK MB 807124

Differs from *Diplocladiella scalaroides* and *Iyengarina elegans* by its holothallic, subhyaline, usually 1-septate conidia.

TYPE: Brazil, Bahia, Pindobaçu, Serra da Fumaça, on decaying leaf of an unidentified monocotyledonous plant, 18.XII.2008, D.A.C. Almeida (**holotype** HUEFS 155081).

ETYMOLOGY: Latin, *elegans*, meaning elegant.

CONIDIOPHORES macronematous, mononematous, grouped, unbranched or branched, cylindrical, erect, straight or flexuous, septate, thick walled, smooth, brown at base, pale brown towards the apex, 22.5–425 × 3.5–6 µm, arising from dark brown, pseudoparenchymatous stromata. CONIDIOGENOUS CELLS terminal, cylindrical, thin-walled, smooth, pale brown, with several enteroblastic percurrent extension which produce fertile dichotomous branches, that form conidia by thallic-arthric disarticulation. CONIDIA thallic-arthric, single, dry, smooth, 1(–3)-septate, asymmetric, branched, Y-shaped,

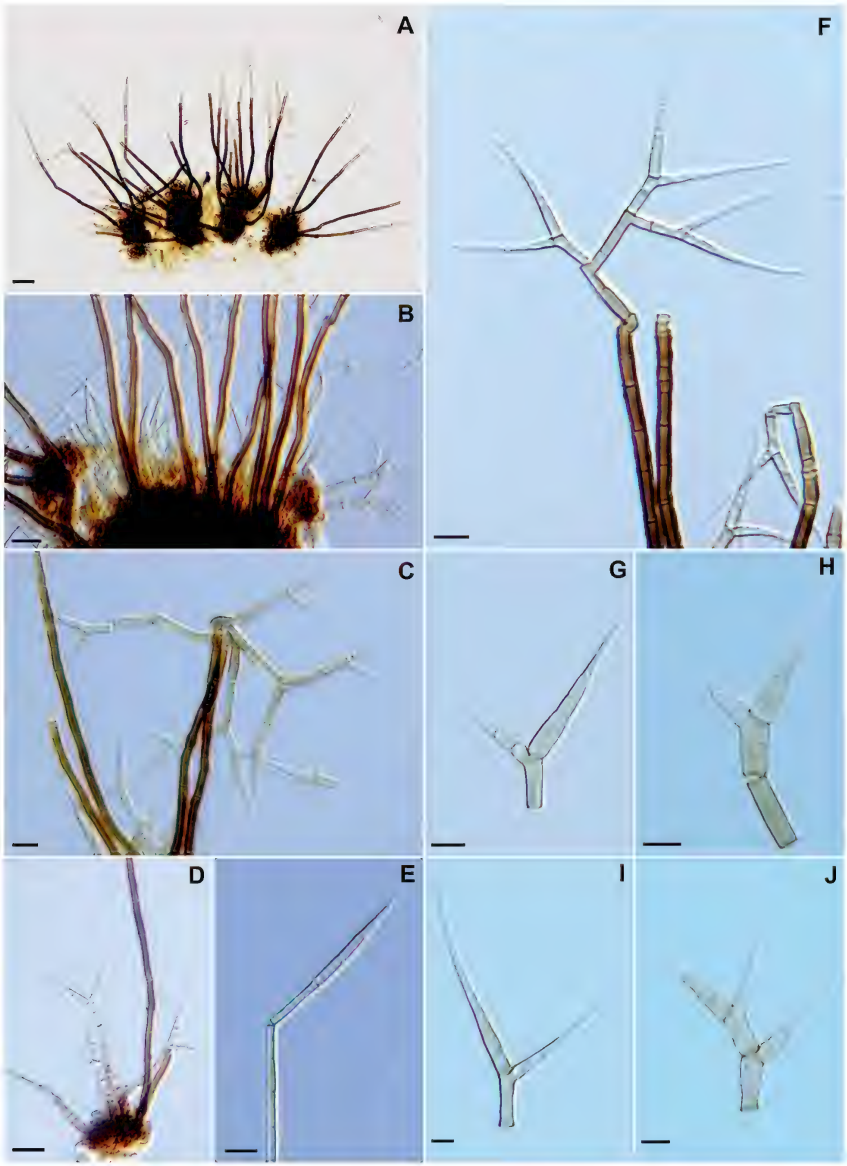


Fig. 1. *Ypsilomyces elegans* (holotype HUEFS 155081): A. Conidiophore and conidia on leaf surface. B. Short conidiophores with conidia and base of long conidiophores. C. Conidiogenous cells with conidia. D. Short conidiophores with conidia. E. Unbranched conidium disarticulating from conidiophore apex. F. Conidiogenous cells with conidia and showing percurrent extension. G–J. Conidia. Scale bars: A = 50 µm; B, D = 20 µm; C = 15 µm; E, F = 10 µm; G–J = 5 µm.

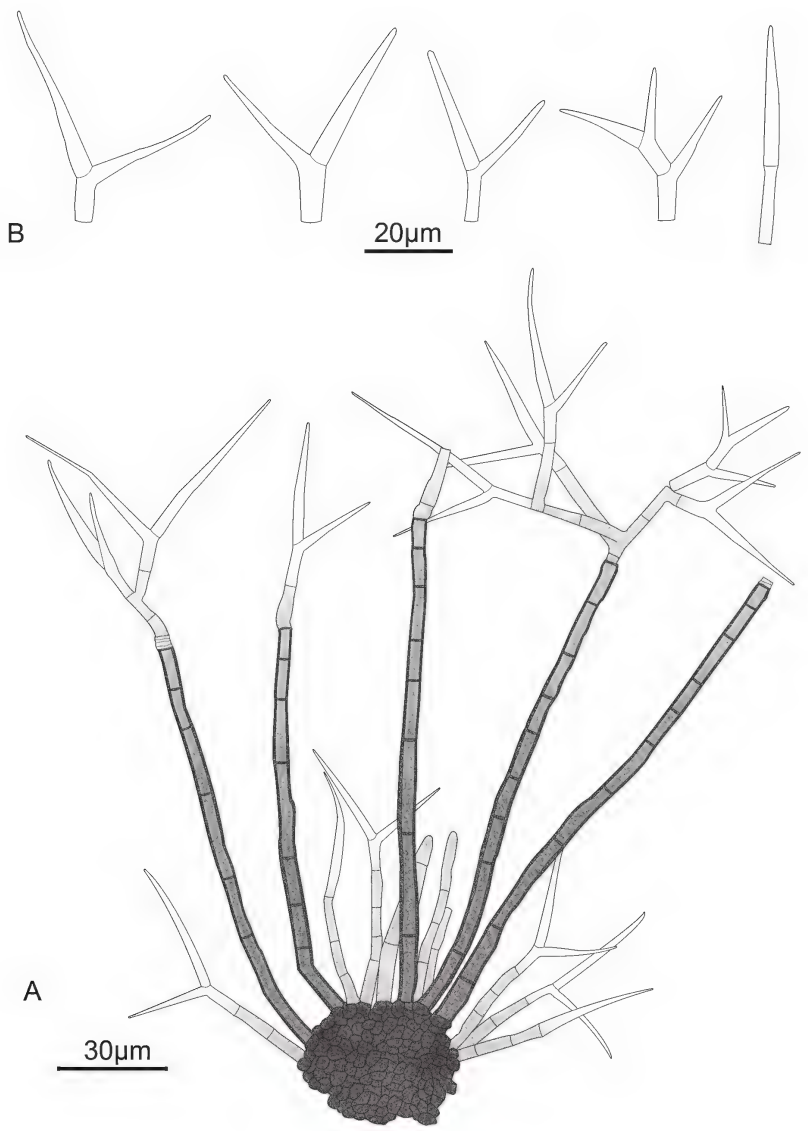


Fig. 2. *Ypsilomyces elegans* (holotype HUEFS 155081): A: General aspect. B: Conidia.

rarely simple or furcate, subhyaline, guttulate, 27–60 × 2.5–5 µm; branches subulate, 14–51 × 2–5 µm.

Comments: Other fungi characterized by Y-shaped conidia include *Biflagellospora* Matsush., *Diademospora* B.E. Söderstr. & Bååth, *Diplocladiella* G. Arnaud ex M.B. Ellis, *Iyengarina* Subram., *Vanterpoolia* A. Funk, *Weufia* Bhat & B. Sutton and *Ypsilina* J. Webster et al. *Ypsilomyces* differs from all these genera by its thallic conidiogenesis and by producing conidiophores on a pseudoparenchymatous stroma. Except for *Weufia* (with tretic conidiogenesis; Bhat & Sutton 1985), all aforementioned genera produce conidia holoblastically. *Vanterpoolia* also produces pseudoparenchymatous stromata, but the stroma is reduced in *Ypsilomyces*, whereas in *Vanterpoolia* it forms a well developed sporodochium (Funk 1982). Moreover, *Vanterpoolia* produces conidia in acropetal chains. *Ypsilomyces* can also be diagnosed by its conidiogenous cells that extend percurrently among successive productions of fertile dichotomous branches, which form thallic-arthric conidia after disarticulation. *Diplocladiella* and *Biflagellospora* differ by the sympodial extension of their conidiogenous cells (Matsushima 1975, Ellis 1976, Sivichai & Hywel-Jones 1999). Although the conidiogenous cells of *Iyengarina* can also extend percurrently, its conidia are produced holoblastically (Kuthubutheen & Nawawi 1992). Other distinctive characteristics of *Ypsilomyces* are the conidiophores that vary greatly in length (22.5–425 µm) and which are pale brown and short when young and basally dematiaceous and long when old. In contrast, *Diademospora* and *Ypsilina* have inconspicuous conidiophores (Söderström & Bååth 1979, Descals et al. 1998).

Acknowledgments

The authors thank Dr. Rafael F. Castañeda Ruíz and Dr. Bryce Kendrick for critically reviewing the manuscript. The authors thank the Program of Research of Biodiversity in the Brazilian Semi-arid (PPBIO Semi-arid/Ministry of Technology and Science – proc. 554718/2009-0) for financial support. The first author thanks the Brazilian Federal Agency for Support and Evaluation of Graduate Education (CAPES) for scholarships and LFPG extends thanks to National Council for Scientific and Technological Development (CNPq proc. 305413/2011-2).

Literature cited

Almeida DAC, Santa Izabel TS, Gusmão LFP. 2011. Fungos conidiais do bioma Caatinga I. Novos registros para o continente americano, Neotrópico, América do Sul e Brasil. Rodriguésia 62(1): 43–53.

Almeida DAC, Barbosa FR, Gusmão LFP. 2012. Alguns fungos conidiais aquáticos-facultativos do bioma Caatinga. Acta Botanica Brasilica 26: 924–932. http://dx.doi.org/10.1590/S0102-33062012000400021

Almeida DAC, Cruz ACR, Marques MFO, Gusmão LFP. 2013. Conidial fungi from the semi-arid Caatinga biome of Brazil. New and interesting *Zanclospora* species. Mycosphere 4(4): 684–692. http://dx.doi.org/10.5943/mycosphere/4/4/4

Barber PA, Keane PJ. 2007. A novel method of illustrating microfungi. Fungal Diversity 27(1): 1–10.

Barbosa FF, Gusmão LFP. 2005. Two *Speiropsis* species (anamorphic fungi–hyphomycetes) from Bahia State, Brazil. Acta Botanica Brasilica 19: 515–518. http://dx.doi.org/10.1590/S0102-33062005000300012

Barbosa FR, Gusmão LFP, Castaneda-Ruiz RF, Marques MFO, Maia LC. 2007. Conidial fungi from the semi-arid Caatinga biome of Brazil. New species *Deightoniella rugosa* and *Diplocladiella cornitumida* with new records for the neotropics. Mycotaxon 102: 39–49.

Barbosa FR, Huzefa AR, Shearer CA, Gusmão LFP. 2013. Some freshwater fungi from the Brazilian semi-arid region, including two new species of hyphomycetes. Cryptogamie, Mycologie. 34 (3): 243–258. http://dx.doi.org/10.7872/crym.v34.iss2.2013.243

Bhat DJ, Sutton BC. 1985. New and interesting hyphomycetes from Ethiopia. Transactions of the British Mycological Society 85(1): 107–122. http://dx.doi.org/10.1016/S0007-1536(85)80160-1

Cruz ACR, Gusmão LFP, Castaneda-Ruiz RF. 2007. Conidial fungi from the semi-arid Caatinga biome of Brazil. *Subramaniomyces pulcher* sp. nov. and notes on *Sporidesmium circinophorum*. Mycotaxon 102: 25–32.

Cruz ACR, Gusmão LFP, Castaneda-Ruiz RF, Stadler M, Minter DW. 2012. *Zelodactylaria*, an interesting new genus from semi-arid northeast Brazil. Mycotaxon 119: 241–248. http://dx.doi.org/10.5248/119.241

Descals E, Marvanová L, Webster J. 1998. New taxa and combinations of aquatic hyphomycetes. Canadian Journal of Botany 76(9): 1647–1659. http://dx.doi.org/10.1139/b98-111

Ellis MB. 1976. More dematiaceous hyphomycetes. Kew, UK, Commonwealth Mycological Institute.

Funk A. 1982. *Vanterpoolia*, a new genus of sporodochial hyphomycetes. Canadian Journal of Botany 60(6): 973–975. http://dx.doi.org/10.1139/b82-122

Gusmão LFP, Leão-Ferreira SM, Marques MFO, Almeida DAC. 2008. New species and records of *Paliphora* from the Brazilian semi-arid region. Mycologia 100(2): 306–309. http://dx.doi.org/10.3852/mycologia.100.2.306

Kuthubutheen AJ, Nawawi A. 1992. New litter-inhabiting hyphomycetes from Malaysia: *Isthmophragmospora verruculosa*, *Iyengarina asymmetrica*, and *Iyengarina furcata*. Canadian Journal of Botany 70: 101–106. http://dx.doi.org/10.1139/b92-014

Matsushima T. 1975. Icones microfungorum a Matsushima lectorum. Kobe, Published by the author.

Santa Izabel TS, Cruz ACR, Gusmão LFP. 2013. Conidial fungi from the semi-arid Caatinga biome of Brazil. *Ellisembiopsis* gen. nov., new variety of *Sporidesmiella* and some notes of *Sporidesmium* complex. Mycosphere 4(2): 156–163. http://dx.doi.org/10.5943/mycosphere/4/2/1

Sivichai S, Hywel-Jones NL. 1999. *Biflagellospora* (aero-aquatic hyphomycetes) from submerged wood in Thailand. Mycological Research 103(7): 908–914. http://dx.doi.org/10.1017/S0953756298007928

Söderström B, Bååth E. 1979. *Diademospora ramigera* gen. et sp. nov. from coniferous forest soil. Transactions of the British Mycological Society 72(2): 340–342. http://dx.doi.org/10.1016/S0007-1536(79)80057-1

Velloso AL, Sampaio EVSB, Pareyn FGC. 2002. Ecorregiões propostas para o Bioma Caatinga. Recife, APNE / Nature Conservancy do Brasil.

ISSN (print) 0093-4666 © 2014. Mycotaxon, Ltd. ISSN (online) 2154-8889

MYCOTAXON

http://dx.doi.org/10.5248/129.187

Volume 129(1), pp. 187–196 *July–September 2014*

Agaricus taeniatus sp. nov., a new member of *Agaricus* sect. *Bivelares* from northwest China

Sai-Fei Li[1,2], Ya-Li Xi[3], Cai-Xia Qi[3], Qian-Qian Liang[3], Sheng-Long Wei[3], Guo-Jie Li[1, 2], Dong Zhao[1], Shao-Jie Li[1A*], & Hua-An Wen[1B*]

[1] *State Key Laboratory of Mycology, Institute of Microbiology, Chinese Academy of Sciences, No 31st Beichen West Road, Chaoyang District, Beijing 100101, China*

[2] *University of Chinese Academy of Sciences, Beijing 100049, China*

[3] *Gansu Engineering Laboratory of Applied Mycology, Hexi University, 846th Huancheng North Road, Ganzhou District, Zhangye 734000, Gansu, China*

* Correspondence to: [A]*lisj@im.ac.cn* [B]*wenha@im.ac.cn*

Abstract—*Agaricus taeniatus* from Qilian Mountains, northwest China, is described as a new species. It is distinguished by its larger basidia and basidiospores, band-like velar remnants on the stipe surface, and persistent single rings with eroded edges. ITS1-5.8S-ITS2 rDNA sequence analyses fully support the establishment of the new species within *A.* sect. *Bivelares*.

Key words —*Agaricales*, *Agaricaceae*, phylogeny, taxonomy

Introduction

Agaricus L. is a genus with nearly 400 species comprising both edible and poisonous species (Bas 1991). It has a long history and has been subdivided into different taxonomic groups (Møller 1950, Heinemann 1978, Cappelli 1984, Singer 1986, Parra 2008). Kauffman (1919) first described *Agaricus* sect. *Bivelares* (Kauffman) L.A. Parra as a section of *Psalliota* typified by *P. rodmanii* (Peck) Kauffman [= *A. bitorquis* (Quél.) Sacc.]. Later studies reorganized this section (Heinemann 1978, Wasser 1980, Challen et al. 2003, Didukh et al. 2004) and characterized its species by negative Schäffer and KOH reactions, red discoloration of the context, and a mild or indistinct odor (Parra 2008). The species most widely consumed, *A. bisporus* (J.E. Lange) Imbach, is included in this section (Kerrigan et al. 2008).

The taxonomy of *Agaricus* has been studied intensively in Europe, America, and southeast Asia (Hotson & Stuntz 1938; Freeman 1979a, 1979b; Heinemann 1956; Kerrigan 1985, 1989, 2005; Chen et al. 2012; Zhao et al. 2012a, 2012b). Despite China's long history of *Agaricus* consumption, its taxonomy remains little studied, and only a few *Agaricus* species have thus far been described from China (Chiu 1973, Li 1990). In our effort to further *Agaricus* taxonomy in the country, we collected specimens from the Qilian Mountains, an area of high macrofungal diversity (Xi et al. 2011). As a result of our research, we present here a new species of *A.* sect. *Bivelares*.

Materials & methods

Photos were taken and main morphological characters were recorded in situ. Other macrocharacters and chemical tests were carried out immediately after the specimens were brought back to the laboratory. Color names and codes follow Ridgway (1912). Specimens were air-dried overnight in a dehydrator prior to microscopic examination. Tissues were hand-sectioned and rehydrated in 5% KOH. Particular attention was paid to the basidia, basidiospores, and cystidia and the anatomy of the pileipellis, annulus, and band-like remnants on the stipe surface. Line drawings were prepared from tissues stained with Congo red. For basidiospores, (*n/m/p*) refers to *n* spores from *m* basidiocarps of *p* specimens, with dimensions given as (a–)b–c(–d), with b–c including 90% of the measured values between the extreme values shown in parentheses. Sterigma lengths are not included. Q = spore length/width quotient in side view; avQ = average Q ± standard deviation. Specimens cited were deposited in Mycological Herbarium, Institute of Microbiology, Chinese Academy of Sciences (HMAS).

DNA extractions were made with a DNA extraction kit (Plant Genomic DNA Rapid Extraction kit, BioTeke, China). Internal transcribed spacers (ITS) were amplified by PCR reactions with primers ITS1/ITS4 (White et al. 1990, Gardes & Bruns 1993), and sequenced by SinoGenoMax Co. Ltd. Sequences obtained were deposited in GenBank.

Sequences of representative and closely related *Agaricus* taxa were retrieved from GenBank. Sequences were aligned using BioEdit (Hall 1999) and MAFFT (http://www.ebi.ac.uk/Tools/msa/mafft/ Katoh & Toh 2010). The alignment has been submitted to TreeBase (submission ID 15563).

Maximum-likelihood (ML) analysis was performed in PhyML3.0 (Guindon et al. 2010), via the ATGC bioinformatics platform (http://www.atgc-montpellier.fr/phyml/). The GTR substitution model was selected with 6 substitution rate categories to account for rate heterogeneity across sites. Likelihood was increased by using the SPR tree improvement. The analysis included 100 bootstrap replicates. Bayesian analyses were performed using MrBayes v3.1.2 (Ronquist & Huelsenbeck 2003) under the estimated evolutionary model. Six simultaneous Markov chains were run for 1,000,000 generations and trees were sampled every 100th generation. The first 2,000 resulting trees were discarded as burn-in and the remaining 8,000 trees were used for calculating the posterior probabilities in the majority rule consensus tree. Trees were visualized in FigTree v1.4.0 (http://tree.bio.ed.ac.uk/software/figtree/).

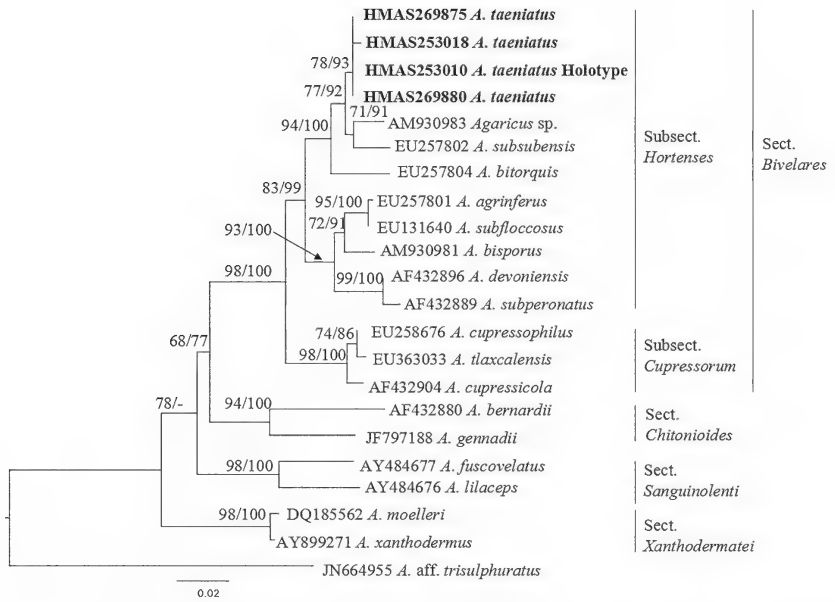


FIG. 1. Phylogenetic tree of *Agaricus* spp. generated from a maximum-likelihood analysis based on the ITS nrDNA sequence. Bootstrap support values (>50%) followed by Bayesian posterior probability values (>50%) are given at the internodes. The tree is rooted by *A.* aff. *trisulphuratus*. Sequences generated in this study shown in bold.

Results

The ITS alignment comprised 22 sequences representing 19 *Agaricus* taxa. *Agaricus* aff. *trisulphuratus* Berk., representing *A.* subg. *Lanagaricus* Heinem., was chosen as outgroup (Zhao et al. 2012b). The ML tree, which showed a topology identical to Bayesian tree, was selected to show the phylogeny of the new species (FIG. 1). The new taxon is well supported with good bootstrap and posterior probability values and is sister to the subclade of *A. subsubensis* and *Agaricus* sp. (AM930983).

Taxonomy

Agaricus taeniatus Sai F. Li, Shao J. Li & H.A. Wen, **sp. nov.** FIGS 2–4

FUNGAL NAMES FN 570120

Differs from other species of *Agaricus* sect. *Bivelares* by its larger basidia and basidiospores, band-like velar remnants on the stipe surface, and persistent single rings with eroded edges.

Fig. 2. *Agaricus taeniatus* habit.
A. HMAS253016; B. HMAS253010 (holotype); C. HMAS269876; D. HMAS269875.

Type: China, Gansu Province, Minle County, Haichaoba, 38°23′50.03″N 100°40′02.90″E, alt. 2593 m, in forest dominated by *Picea crassifolia*, 10 August 2013, S.F. Li, Y.L. Xi, C.X. Qi & Q.Q. Liang QLS60 (**Holotype**, HMAS253010; GenBank KJ623317); QLS15 (**Isotype**, HMAS253017; GenBank KJ623319).

Etymology: refers to the band-like velar remnants on the stipe surface.

Pileus 6–17 cm diam., irregularly hemispheric with a flat top when young, then expanding to plano-convex, and finally applanate and marge wavy; surface covered with fibril or fibrillose squamules, adpressed, Cinnamon (XXIX15″) to Mikado Brown (XXIX13″i) upon a barely discernible background, squamules patch-like on the disc, decreasing in size towards the margin; edge appendiculate; no discoloration on bruising. Context 7–20 mm thick from the centre of pileus to margin, firm, white, becoming Orange-Pink (II11f) at the disc, Jasper Red (XIII3′) at the lamellar/context interface. Lamellae free, 6–8 mm broad, 13–24/cm at the edge, white when young, maturing from light pink, light brown, brown and to finally dark brown. Annulus 6–17 mm broad, persistent, single, thick near the stipe and thinner toward an eroded edge, upper surface striate, lower surface fibrillose-woolly, having obvious cortinate fibrils

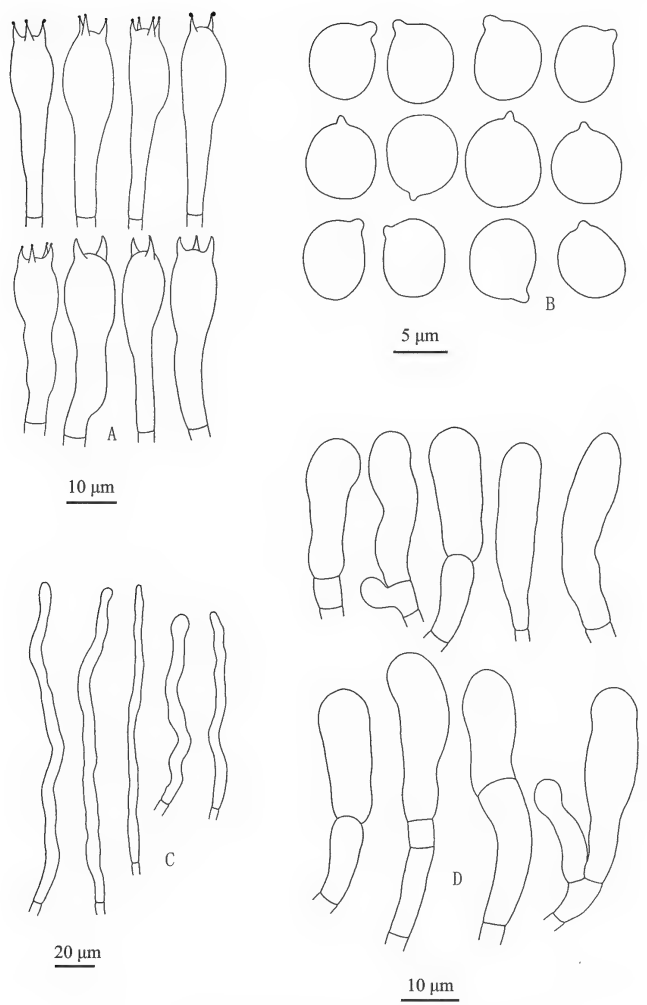


Fig. 3. *Agaricus taeniatus* (HMAS253010, holotype).
A. Basidia; B. Basidiospores; C. Hyphae from band-like remnants; D. Cheilocystidia.

at the stipe, generally superior, white. Stipe 2–6 × 7–22 cm, cylindrical, ± equal or (sometimes) distinctly bulbous with pointed base, solid, surface covered with small, appressed squamules above annulus, heavily floccose-fibrillose below annulus, white overall, Eosine Pink (I1d) around some squamules, bruising Ferruginous (XIV9′i); veils decorating stipe in a series of complete or partial transverse bands concolorous with the surface squamules, discernible when young; context white, becoming Vinaceous-Rufous (XIV7′i) when cut at

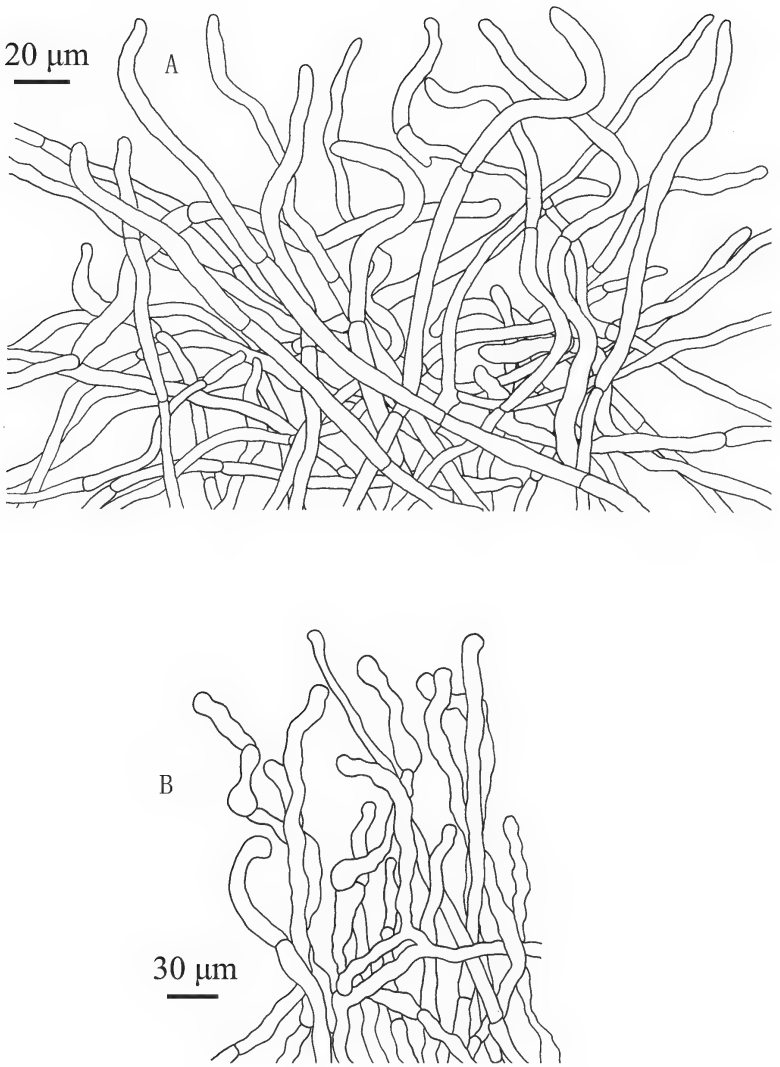


Fig. 4. *Agaricus taeniatus* (HMAS253010, holotype).
A. Hyphae from pileipellis; B. Hyphae from annulus.

the stipe base of stipe, Pale Flesh (XIV7′f) above base. Odor indistinct. Taste pleasant.

Macrochemical reactions: KOH and Schäffer reaction negative.

Basidiospores [120/8/8] (5.1–)5.7–7.1(–8.4) × (6.1–)6.8–8.6(–10.7) µm, average 6.3 × 7.6 µm, Q = (1.04–)1.11–1.30(–1.38), avQ = 1.20 ± 0.07, subglobose to broadly ellipsoid, without germ pore, thick-walled, smooth, dark brown when mature. Basidia 8–11(–12) × (32–)33–41(–42) µm, clavate, 4-spored; sterigmata 2.5–4.6 µm long. Cheilocystidia clavate to cylindrical, sometimes flexuose, usually septate with 1–3 basal septa, hyaline, smooth; terminal elements measuring (5–)6–9(–10) × (16–)18–38(–45) µm. Pleurocystidia absent. Pileipellis a cutis, composed of hyphae 3–14 µm diam., cylindrical, hyaline, smooth, not or slightly constricted at the septa; terminal cells 3–9 × 33–154 µm, slightly attenuated toward apex. Annulus hyphae 4–14 µm diam., cylindrical or irregularly cylindrical, inflated or constricted at some places of the hyphae, hyaline, smooth, not constricted, slightly constricted, or inflated at the septa; terminal cells 4–16 × 39–270 µm, slightly or sometimes obviously inflated toward apex. Band-like remnants composed of hyphae 2.3–6.6 µm diam., cylindrical, hyaline, smooth; terminal cells 4–9 × 83–163 µm, slightly attenuated toward apex. Clamp connection not observed.

Habit: Scattered or solitary in fluffy soil covered with lush moss in *Picea crassifolia* Kom. forest.

> Additional specimens examined: **China, Gansu Province,** Minle County, Haichaoba, 38°23′50.03″N 100°40′02.90″E, alt. 2593 m, 6 August 2013, S.F. Li, Y.L. Xi, C.X. Qi & Q.Q. Liang QLS04 (HMAS253016; GenBank KJ623318); Sunan County, Kangle, 38°48′22.02″N 99°55′58.57″E, alt. 2766 m, 8 August 2013, S.F. Li, Y.L. Xi, C.X. Qi & Q.Q. Liang QLS20 (HMAS269876; GenBank KJ623321); QLS21 (HMAS269875; GenBank KJ623322); QLS28 (HMAS253018; GenBank KJ623323); Haichaoba, 10 August 2013, S.F. Li, Y.L. Xi, C.X. Qi & Q.Q. Liang QLS64 (HMAS269880; GenBank KJ623320); QLS65 (HMAS253019; GenBank KJ623324).

Discussion

The brownish pileus squamules, superior annulus with veil remnants, reddish discoloration when cut, indistinct odor, and negative KOH and Schäffer reactions place *A. taeniatus* in *A.* subsect. *Hortenses* Heinem. (Kerrigan et al. 2008), which the ITS-based phylogeny supports.

Among the species of this section, the squamulose pileus and single annulus of *A. taeniatus* easily separate it from *A. bitorquis* and *A. devoniensis* P.D. Orton, both of which have a smooth pileus surface and double rings. *Agaricus subperonatus* (J.E. Lange) Singer, which shares similar pileus characters and a stipe with complete or incomplete transverse bands, differs from *A. taeniatus* by forming a double-edged annulus that is <5 mm broad and slightly fibrillose on the underside (Bohus 1975, Parra 2008).

A stipe decorated with heavily floccose-fibrillose squamules and a series of complete or incomplete transverse bands are characters that easily separate *A. taeniatus* from other species of *A.* subsect. *Hortenses* characterized by a

relatively simple stipe surface (e.g., *A. maleolens* F.H. Møller, *A. subfloccosus* (J.E. Lange) Hlaváček, *A. agrinferus* Kerrigan & Callac, and *A. tlaxcalensis* Callac & G. Mata). Additional differences are: *A. maleolens* has stems with few ochraceous spots or small ochre squamules towards the base (Cappelli 1984, Lacheva & Stoichev 2004); *A. subfloccosus* occasionally has sparse woolly remnants on glabrous stipes (Parra 2008); *A. agrinferus* has glabrous to minutely floccose stipes sometimes with fibrillose zones below (Kerrigan et al. 2008); and *A. tlaxcalensis* has a longitudinally fibrillose and silky stipe surface (Kerrigan et al. 2008).

Agaricus cupressicola Bon & Grilli has been placed into different sections: either *A.* sect. *Bivelares* based on phylogenetic analysis (Kerrigan 2008) or *A.* sect. *Sanguinolenti* based on morphology (Parra 2008). This species differs from *A. taeniatus* by its much smaller basidiocarps, relatively strongly rufescent context, and thin annulus with an almost smooth lower surface.

Microscopically, *A. taeniatus* is easily separated from *A. bisporus* by its 4-spored basidia and from *A. cupressophilus* Kerrigan (3.7–3.9 × 4.6 µm) and *A. devoniensis* subsp. *bridghamii* Kerrigan (5.0–5.5 × 5.9–6.6 µm) by its much larger spores and longer cheilocystidia (19–23 µm in the two latter taxa). *A. cupressophilus* and *A. devoniensis* subsp. *bridghamii* are further distinguished by smaller pilei (3–7.5 cm diam.) and shorter stipes (1–2 × 4–8 cm) (Kerrigan et al. 2008). *Agaricus subedulis* Heinem. differs from *A. taeniatus* in forming shorter (<33 µm) basidia, wider (>9 µm) cheilocystidia, and irregularly shaped cheilocystidia with constricted ends (Heinemann 1956).

The phylogeny (Fig. 1) shows *A. taeniatus* most closely related to *A. subsubensis* Kerrigan and *Agaricus* sp. (AM930983). *Agaricus taeniatus* resembles *A. subsubensis* in the brownish pileus, reddish discoloration when sectioned and a series of thin broken ring-like remnants on the stipe. However, *A. subsubensis* has shorter (24–30 µm) basidia, a much less robust (0.5–1.5 × 0.5–4.5 cm) stipe, and a much more narrow, indistinct, band-like annulus (Kerrigan et al. 2008). *Agaricus* sp. (AM30983), collected from Lake Baikal district in southern Siberia, Russia, has been tentatively identified as *A. balchaschensis* Samgina & G.A. Nam but lacks sufficient taxonomic data for a positive identification (Hildén et al. 2013); *A. balchaschensis* differs from *A. taeniatus* in forming a stouter stipe (7–8 × 10–14 cm), movable annulus, a context that more strongly discolors (orange to cherry red) upon exposure, and shorter (23–34 µm) basidia (Samgina & Nam 1989).

Acknowledgments

The authors are grateful to Dr. R.L. Zhao and Dr. Kanad Das for reviewing the manuscript; to Dr. R.W. Kerrigan and L.A. Parra for providing phylogenetic and documental information of related species; to Dr. X.G. Zhang for improving the manuscript; to Ms. H.M. Lü, Ms. A.R. Yan and Dr. T.Z. Wei for providing the herbarium

specimens; to Ms. L.T. Zhang for assistance in lab work; to Mr. L. Su, Ms. Y.M. Li for assistance in phylogenetic analyses.

Literature cited

Bas C. 1991. A short introduction to the ecology, taxonomy and nomenclature of the genus *Agaricus*. 21–24, in: LJLD van Grievsen (ed.). Genetics and breeding of *Agaricus*. Proceedings of the First International Seminar on Mushroom Science. Wageningen, Pudoc.

Bohus G. 1975. *Agaricus* studies, V. (*Basidiomycetes, Agaricaceae*). Annales Historico Naturales Musei Nationalis Hungarici 67: 37–40.

Cappelli A. 1984. *Agaricus* L.: Fr. (*Psalliota* Fr.). Saronno. Libreria editrice Biella Giovanna.

Challen M, Kerrigan RW, Callac P. 2003. A phylogenetic reconstruction and emendation of *Agaricus* section *Duploannulatae*. Mycologia 95(1): 61–73. http://dx.doi.org/10.2307/3761962

Chen J, Zhao RL, Karunarathna SC, Callac P, Raspé O, Bahkali AH, Hyde KD. 2012. *Agaricus megalosporus*: a new species in section *Minores*. Cryptogamie Mycologie 33(2): 145–155. http://dx.doi.org/10.7872/crym.v33.iss2.2012.145

Chiu WF. 1973. Ten new species of agarics from Yunnan. Acta Microbiology Sinica 13(2): 129–135.

Didukh M, Wasser SP, Nevo E. 2004. New data on rare and debatable species of the family *Agaricaceae* (Fr.) Cohn. Ukrayins'k. Bot. Zhurn. 61(1): 89–99.

Freeman AEH. 1979a. *Agaricus* in North America: type studies. Mycotaxon 8: 1–49.

Freeman AEH. 1979b. *Agaricus* in the southeastern United States. Mycotaxon 8: 50–118.

Gardes M, Bruns TD. 1993. ITS primers with enhanced specificity for basidiomycetes-application to the identification of mycorrhizae and rusts. Molecular Ecology 2: 113–118. http://dx.doi.org/10.1111/j.1365-294X.1993.tb00005.x

Grilli E. 1988. Il genre *Agaricus* L.: Fr. in Abruzzo, II. *Agaricus cupressicola* Bon & Grilli. Micologia e Vegetazione Mediterranea 3(2): 111–129.

Guindon S, Dufayard JF, Lefort V, Anisimova M, Hordijk W, Gascuel O. 2010. New algorithms and methods to estimate maximum-likelihood phylogenies: assessing the performance of PhyML 3.0. Systematic Biology 59(3): 307–321. http://dx.doi.org/10.1093/sysbio/syq010

Hall TA. 1999. BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. Nucleic Acids Symposium Series 41: 95–98.

Heinemann P. 1956. Champignons récoltés au Congo belge par Mme M. Goossens-Fontana, II. *Agaricus* Fr. s.s. Bull. Jard. Bot. l'Etat Bruxelles 26: 1–127.

Heinemann P. 1978. Essai d'une clé de determination des genres *Agaricus* et *Micropsalliota*. Sydowia 30: 6–37.

Hildén K, Mäkelä MR, Lankinen P, Lundell T. 2013. *Agaricus bisporus* and related *Agaricus* species on lignocellulose: production of manganese peroxidase and multicopper oxidases. Fungal Genetics and Biology 55: 32–41. http://dx.doi.org/10.1016/j.fgb.2013.02.002

Hotson JW, Stuntz DE. 1938. The genus *Agaricus* in western Washington. Mycologia 30: 204–234. http://dx.doi.org/10.2307/3754557

Katoh K, Toh H. 2010. Parallelization of the MAFFT multiple sequence alignment program. Bioinformatics 6(15): 1899-1900. http://dx.doi.org/10.1093/bioinformatics/btq224

Kauffman CH. 1919. The *Agaricaceae* of Michigan. Lansing MI, Wynkoop Hallenbeck Crawford Co., State Printers. http://dx.doi.org/10.5962/bhl.title.58545

Kerrigan RW. 1985. Studies in *Agaricus* III: new species from California. Mycotaxon 20: 419–434.

Kerrigan RW. 1989. Studies in *Agaricus* IV: new species from Colorado. Mycotaxon 34: 119–128.

Kerrigan RW. 2005. *Agaricus subrufescens*, a cultivated edible and medicinal mushroom, and its synonyms. Mycologia 97: 12–24. http://dx.doi.org/10.3852/mycologia.97.1.12

Kerrigan RW, Callac P, Parra LA. 2008. New and rare taxa in *Agaricus* section *Bivelares* (*Duploannulati*). Mycologia 100(6): 876–892. http://dx.doi.org/10.3852/08-019

Lacheva MN, Stoichev GT. 2004. New species of the genus *Agaricus* (*Agaricaceae*) for Bulgaria. Mycologia Balcanica 1: 35–40.

Li Y. 1990. New species and records of the genus *Agaricus* from China. Acta Botanica Yunnanica 12(2): 154–160.

Møller FH. 1950. Danish *Psalliota* species, I–II. Friesia 4: 1–60.

Parra LA. 2008. *Agaricus* L.: *Allopsalliota* Nauta & Bas. Fungi Europaei, vol. 1. Alassio, Edizioni Candusso.

Ridgway R. 1912. Color standards and nomenclature. Washington, Robert Ridgway. http://dx.doi.org/10.5962/bhl.title.62375

Ronquist F, Huelsenbeck JP. 2003. MrBayes 3: Bayesian phylogenetic inference under mixed models. Bioinformatics 19: 1572–1574. http://dx.doi.org/10.1093/bioinformatics/btg180

Samgina DI., Nam GA. 1989. Novyĭ vid iz roda *Agaricus* L.: Fr. Izvestiya Akademii Nauk Kazakhskoĭ SSR, Seriya Biologicheskaya 156: 78–80.

Singer R. 1986. The *Agaricales* in modern taxonomy. 4th ed. Koenigstein, Koeltz Scientific Books. http://dx.doi.org/10.2307/1219523

Wasser SP. 1980. Flora Fungorum RSS Ucrainicae. Kiev, Naukova Dumka.

White TJ, Bruns T, Lee S, Taylor J. 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. 315–322, in: MA Innis et al. (eds). PCR protocols: a guide to methods and applications. San Diego, Academic.

Xi YL, Wang ZJ, Yu HP, Wei SL. 2011. Preliminary research on macro-fungi of Qilian Mountain National Nature Reserve. Edible Fungi of China 30(4): 7–13.

Zhao RL, Desjardin DE, Callac P, Parra LA, Guinberteau J, Soytong K, Karunarathna S, Zhang Y, Hyde KD. 2012a. Two species of *Agaricus* sect. *Xanthodermatei* from Thailand. Mycotaxon 122: 187–195. http://dx.doi.org/10.5248/122.187

Zhao RL, Hyde KD, Desjardin DE, Raspé O, Soytong K, Guinberteau J, Karunarathna S, Callac P. 2012b. *Agaricus flocculosipes* sp. nov., a new potentially cultivatable species from the palaeotropics. Mycoscience 53: 300–311. http://dx.doi.org/10.1007/s10267-011-0169-5

ISSN (print) 0093-4666 © 2014. Mycotaxon, Ltd. ISSN (online) 2154-8889

MYCOTAXON

http://dx.doi.org/10.5248/129.197

Volume 129(1), pp. 197–208 *July–September 2014*

Macrolepiota distribution extends to the montane temperate forests of Pakistan

Muhammad Fiaz[1], Sana Jabeen[2*], Amna Imran[2], Habib Ahmad[3], & Abdul Nasir Khalid[2]

[1]*Department of Botany &* [3]*Department of Genetics, Hazara University, Mansehra, Pakistan*

[2]*Department of Botany, University of the Punjab, Quaid-e-Azam Campus-54590, Lahore, Pakistan*

* Correspondence to: *ectomycorrhizae@gmail.com*

Abstract — Basidiomata of *Macrolepiota dolichaula* and *M. excoriata* were collected from mixed coniferous forests in Pakistan. The species were identified using ITS nrDNA sequence analyses and morphological and anatomical characters. Detailed morphological and anatomical descriptions and illustrations are provided along with results of molecular phylogenetic analysis. Both species are regarded as new records for Pakistan, and this report considerably extends their known distribution.

Key words — *Agaricaceae*, basidiomata, *Basidiomycota*, saprotrophic

Introduction

Macrolepiota was established by Singer (1948). Currently, there are about 33 species recognized in the genus worldwide (Kirk et al. 2008; Ge et al. 2010, 2012), of which several are edible (Ding & Huang 2003). Representatives of the genus typically have large fleshy basidiomata, a pileal surface covered by squamules composed of long subcylindric elements, white to cream lamellae, and a prominent annulus. The basidiospores are relatively big and thick-walled with a germ pore covered by a hyaline cap, and stipe squamules are often visible as colored bands in mature specimens (Singer 1948; Vellinga 2003; Vellinga et al. 2003). Some *Macrolepiota* species have been transferred to *Chlorophyllum* Massee, which differs from *Macrolepiota* s. str. by the presence of pileal squamules made up of a hymenodermal layer, spore germ pores that are either absent or truncated, and a smooth stipe (Vellinga & Kok 2002).

Macrolepiota species are saprotrophic and grow in the soil litter layers that contain mostly fresh leaves (Vellinga 2004). Some species, such as *M. excoriata* and *M. phaeodisca* Bellù, have a restricted distribution (Courtecuisse & Duhem 1994; Nauta & Vellinga 1995; Candusso & Lanzoni 1990), although the exact distribution is still unknown for many species. *Macrolepiota procera* (Scop.) Singer is the only species that has been reported from Pakistan (Ahmad et al. 1997).

The present study focuses on *M. dolichaula* and *M. excoriata*, newly recorded from Pakistan from two different areas of Khyber Pakhtunkhawa (KPK) Province. *Macrolepiota dolichaula* has been previously reported from China (Chiu 1948; Shao & Xiang 1981; Zang et al. 1996; Bi et al. 1994, 1997; Ge et al. 2010, 2012), East Africa (Pegler 1977), eastern Australia (Grgurinovic 1997; Vellinga 2003), India (Manjula 1983; Natarajan & Manjuk 1983), Sri Lanka (Pegler & Rayner 1969), Thailand (Ge et al. 2010), and Vietnam (Kiet 1998; Yang 2000). *Macrolepiota excoriata* is native to Europe and has also been reported from India (Kumari 2012), Israel (Barseghyan et al. 2012), South Africa (Doidge 1950), Turkey (Afyon & Yağiz 2004; Doğan & Öztürk 2006), China (Teng 1996; Mao 2000; Ge et al. 2010), and North America and South Africa (http://www.gbif.org/). The correct identity of some of these records, however, requires confirmation.

Materials & methods

Sampling site description

The two sampling sites are situated in the Mansehra and Swat districts of Khyber Pakhtunkhwa.

Mansehra district is located at the eastern border of the KPK. Mountain ranges, plains, valleys, and numerous lakes and rivers are the main features, with the Kunhar and Siran rivers among the largest in the area. The climate is warm in summer and cold in winter, although the northern high mountainous region remains cold in summer due to snow cover. Evergreen and deciduous trees are well represented. *Pinus wallichiana* A.B. Jacks. and *Pinus roxburghii* Sarg. are dominant tree species, while *Juglans regia* L. is the most common broadleaf tree (Mustafa 2003).

The Swat valley, which lies in northern Khyber Pakhtunkhwa, is surrounded by mountains that are offshoots of the Hindu Kush. The Swat River is formed by the joining of the Ushu and Utrot rivers, which descend from the Hindu Kush Mountains. Kalam, a village along the upper reaches of the Swat River, is known for its waterfalls, lakes, and lush green hills dominated by *Cedrus deodara* (Roxb. ex D. Don) G. Don forests, *Pinus* spp., and *Quercus* sp. The climate is moist temperate to dry temperate (Champion et al. 1965). Following the standard classification of Pakistan's forests (Champion et al. 1965), Shah & Khan (2006) classify the Swat forest type as belonging to "montane temperate forest."

Morphological & anatomical characterization

Fresh basidiomata were collected and catalogued, described morphologically, and photographed using a Nikon D70S digital camera. Color codes follow Munsell Soil Color Charts (1975). The specimens were dried under a fan heater. The specimens were mounted in 5% KOH solution for examination of basidia, cystidia, spores, pileal elements and stipe hyphae under a MX4300H microscope (Meiji Techno Co., Ltd., Japan). Anatomical features were measured with a Carl Zeiss Jena ocular micrometer. Basidiospore dimensions follow the format [*n/m/p* = *n* basidiospores measured from *m* fruit bodies of *p* collections], length × width (l × w), and avQ (= average l/w ratio of all spores). Line drawings were made using Leitz Wetzlar Camera Lucida. Voucher specimens were deposited in either the Herbarium, Department of Botany, University of the Punjab, Lahore, Pakistan (LAH) or the Herbarium Hazara University, Dhodial, Pakistan (HUP).

Molecular characterization

A small piece (2 mg) of lamellae was kept in 2% CTAB buffer. DNA was extracted using modified CTAB method following Bruns (1995). The nrDNA ITS regions were amplified using ITS1F (CTTGGTCATTTAGAGGAAGT) and ITS4 (TCCTCCGCTTATTGATATGC) primers under standard conditions (Gardes & Bruns 1993) Agarose Gel Electrophoresis was performed to visualize PCR products in Gel Documentation System (UVtec. Avebury House, Cambridge CB4 1QB UK) at default settings. The products were sent to Macrogen Inc. (Korea) to perform sequencing using the same pair of primers. The consensus sequence was generated from the sequences obtained using both primers. A BLAST search was used to retrieve nucleotide sequences from GenBank (http://www.ncbi.nlm.nih.gov/), with the closest matching sequences selected from the initial blast. The most closely related species were also included in the final data set. ClustalW was used to align sequences in MEGA software. The sequences were trimmed with the conserved motifs 5′-(...GAT)CATTA... and ...GACCT(CAAA...)-3′, and the alignment portion between them was included in phylogenetic analysis. Maximum likelihood (ML) analysis was performed using Jukes-Cantor model and nearest neighbor interchange as ML heuristic search method in MEGA5 software to test the phylogeny at 1000 bootstraps. The rDNA-ITS divergence was reconstructed using MegAlign (DNAStar). Sequences generated in this study were submitted to GenBank (KJ013326; KJ643333; KJ643334).

Taxonomy

Macrolepiota dolichaula (Berk. & Broome) Pegler & R.W. Rayner, Kew Bull. 23: 365 (1969). Fig. 1

Basidiomata medium to large with long stipe. Pileus ≤13.5 cm diam., fleshy, convex to plano-convex, white (5Y9/2), with inwardly projected margins, umbonate, covered with minute, pallid, yellowish brown (10YR5/6) squamules, crowded at centre, become minute and sparse towards margins; disc yellowish brown (10YR5/6); margins rough, fragile at maturity; context thick and white (5Y9/2). Lamellae free, crowded, white (5Y9/2) when young, becoming off-

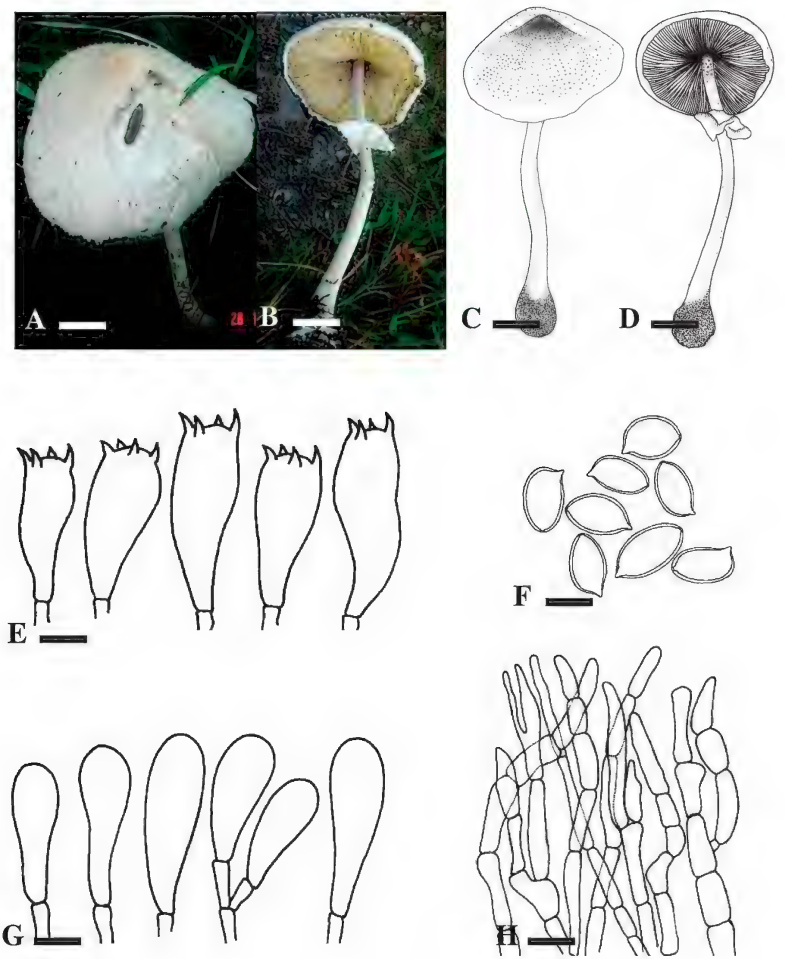


Figure 1: *Macrolepiota dolichaula* (FM-22). A–D. Basidiomata; E. Basidia; F. Basidiospores; G. Cheilocystidia; H. Hyphae from pileus. Scale bars: A = 3 cm; B–D = 4.5 cm; E = 13 µm; F = 9 µm; G = 10 µm; H= 15 µm.

white to cream (2.5Y8/4) at maturity. Lamellulae few in general. Stipe ≤26 cm long, gradually tapering towards the apex, diam. 0.5 cm towards the pileus and ≤1.3 cm towards the base, farinose, mostly glabrous, off-white to cream (2.5Y8/4), scabrous just below the annulus, with brown (10YR7/6) scales; base bulbous, ≤2 cm in diam. Annulus ascending, whitish (2.5Y8/4), superior, membranous, moveable. Odor and taste not recorded.

Basidiospores [50/1/1] 9.4–15.6 × 7.5–11.3 µm (average = 12.5 × 9.4 µm), avQ = 1.33, ovoid to broadly ellipsoid, ellipsoid, hyaline in 5% KOH, reddish brown in Melzer's reagent, with a germ pore on the rounded apex with a hyaline cap; apiculus 1–1.5 µm high. Basidia 29–39.6 × 9.9–14.4 µm, clavate to subclavate, thin-walled, hyaline in 5% KOH, 4-spored. Pleurocystidia absent. Cheilocystidia 18.4–28.4 × 7.1–12.8 µm, clavate, thin walled, hyaline. Squamules on pileus consisting of cylindric to subcylindric, branched, clampless hyphae, 3–10 µm in diam., terminal elements cylindric to slightly attenuating towards the tip, hyaline to yellowish brown vacuolar pigment. Clamp connections occasional at basidial bases.

Material examined—**PAKISTAN**, **Mansehra District**, Khabbal Paien, 1450 m asl, on soil under *Pinus wallichiana*, August 2009, Muhammad Fiaz FM–22 (HUP MFM–331; GenBank KJ643334).

Macrolepiota excoriata (Schaeff.) Wasser, Ukr. Bot. Zh. 35: 516 (1978) Fig. 2

Basidiomata medium to large. Pileus ≤8 cm in diameter, fleshy, convex to plano-convex with smooth margins, slightly umbonate, white to off white (2.5Y8/4), covered with furfuraceous brownish squamules (10YR5/6), which become sparse toward margin; disc smooth, brownish (10YR5/6), margins rough and fragile. Lamellae free, ≤9 mm broad, crowded with 1–2 tiers of short lamellulae, white to off white (2.5Y8/4). Stipe 7.3–14.5 cm long, diam. 0.4 cm towards the point of attachment and 0.7 cm towards the base, with abruptly bulbous base ≤1.7 cm diam., central, hollow, fibrillose, white to off white (2.5Y8/4), cylindrical, white to off-white (2.5Y8/4), covered with minute brownish (10 YR 5/6) squamules above the annulus. Annulus persistent, descending, whitish (2.5Y8/4), membranous, superior, about 3 cm below the stipe apex. Context white, not changing when bruised. Odor and taste not recorded.

Basidiospores [50/2/2] 12.7–18.4 × 11.1–12 µm (average = 15.7 × 11.5 µm), avQ = 1.36, ellipsoid to ovoid with one or many guttules, thick-walled (0.8 µm), smooth, off-white or pale yellow in 5% KOH, light brown to reddish brown in Melzer's reagent, with rounded apex; germ pore visible; apiculus prominent. Basidia 40.3–47.4 × 16.0–18.6 µm (average = 43.6 × 17.2 µm), at base 5.7 µm wide on average, clavate, guttulate, thin-walled, commonly 4-spored, hyaline to off-white or pale yellow in KOH; sterigmata 6 µm long. Pleurocystidia absent. Cheilocystidia 29.3–32.1 × 11.1–13.9 µm (average = 31 × 11.9 µm), base 5 µm, thin-walled, hyaline to off-white or pale yellow in 5% KOH, clavate. Squamules made up of filamentous, branched, cylindrical, clampless, hyphae 3.3–16 µm in diam. with yellowish pigment; terminal segments 32.5–153.4 × 6.3–12.5 µm. Stipe hyphae thin walled, hyaline to cream. Clamp connections not observed.

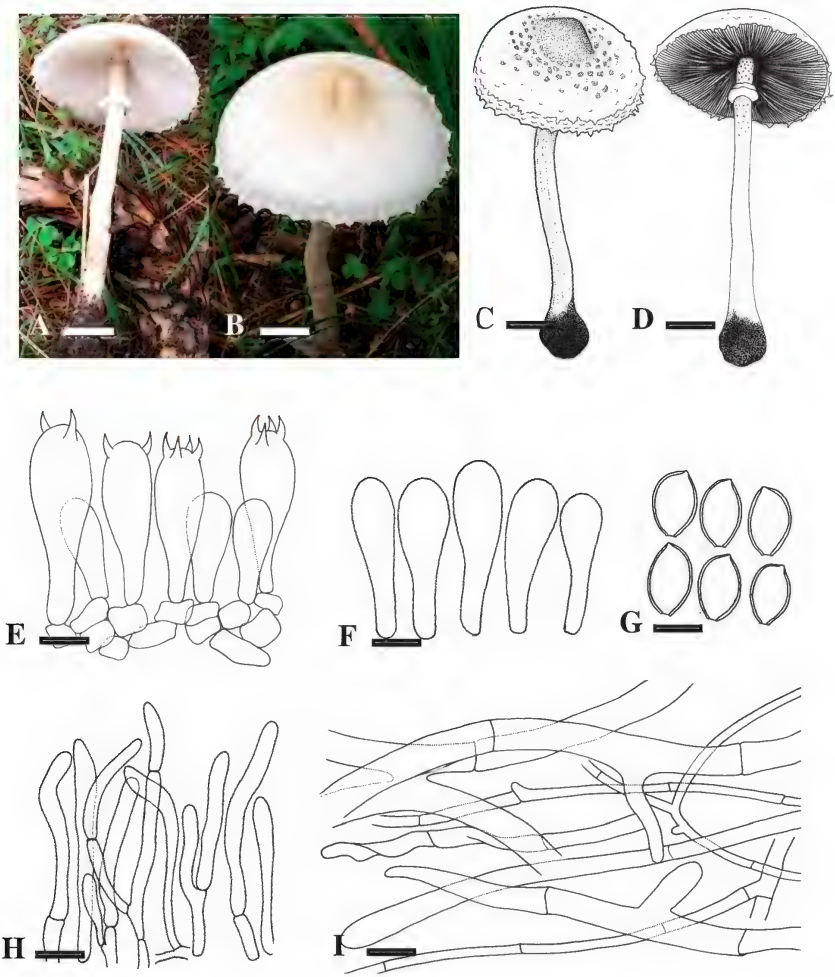


FIGURE 2: *Macrolepiota excoriata* (FM-305 [A–D]; F2; K3-1 [E–I]). A–D. Basidiomata; E. Basidia; F. Cheilocystidia; G. Basidiospores; H. Hyphal squamules; I. Hyphae from pileipellis. Scale bars: A, C, D = 3 cm; B = 2 cm; E = 10 µm; F = 9 µm; G = 8 µm; H = 18 µm; I = 21 µm.

MATERIAL EXAMINED–**PAKISTAN**, **MANSEHRA DISTRICT**, Khabbal Paien, Dub, 1700 m asl, on soil under *Pinus wallichiana*, July 2009, Muhammad Fiaz FM–305 (HUP MFM–332; GenBank KJ643333); **SWAT DISTRICT**, Kalam, 2070 m asl, on soil under *Cedrus deodara*, 4 September 2013, Sana Jabeen F2; K3-1 (LAH-SJ1-2013; GenBank KJ013326).

Phylogeny

The nrITS sequences of *Macrolepiota excoriata* comprised 750–753 (ITS1F primer) and 760–769 (ITS4 primer) base pairs; a consensus sequence of 654 base pairs was obtained by trimming the motif of both sequences. *Macrolepiota dolichaula* sequences comprised 1390 (ITS1F primer) and 768 (ITS4 primer) base pairs. The BLAST of *M. excoriata* (KJ013326) revealed that it was 99% identical to JQ683100, JQ683089, HM246504, AF482840, AY243606, and JQ683118 with 100% query cover and 0.0 E value; *M. dolichaula* was100% identical to AF482839 with 100% query cover and 0.0 E value. Published sequences from different *Macrolepiota* sections were included to reconstruct the phylogeny with *Leucoagaricus barssii* (Zeller) Vellinga as outgroup. The analysis revealed three major clades within *Macrolepiota* (Fig. 3; /macrolepiota, /macrosporae, /volvatae), which correspond to sections *Macrolepiota*, *Macrosporae*, and *Volvatae* in earlier studies (Vellinga et al. 2003; Ge et al. 2010). Within /macrolepiota, *M. dolichaula*, *M. detersa*, *M. procera*, *M. colombiana*, *M. fuliginosa*, *M. subcitrophylla* and *M. clelandii* clustered with 58% bootstrap support; within /macrosporae, *M. phaeodisca*, *M. konradii*, *M. mastoidea*, *M. subsquarrosa*, *M. orientiexcoriata*, and *M. excoriata* clustered with 97% bootstrap support; and within /volvatae, the two volvate species, *M. eucharis* and *M. velosa,* clustered with 100% bootstrap support.

Discussion

Macrolepiota is a monophyletic group in accordance with ITS based findings (Vellinga et al. 2003). In present study, all three clades recovered by the ITS data set received strong bootstrap support. Morphological characters also support separation of these clades.

Macrolepiota dolichaula (KJ643334) from Pakistan clustered with similar specimens in sect. *Macrolepiota,* which is characterized by a complex annulus, relatively big (usually 14–20 µm) ovoid-ellipsoid spores, frequent clamp connections at the bases of the cheilocystidia and basidia, and a long stipe (Bon 1996). Within this section, the stipe surface usually has fine brown squamules, but *Macrolepiota dolichaula* with a farinose stipe surface is an exception. The pileus within this section usually forms plate-like squamules, but again *M. dolichaula* is an exception, with its pileus covered by minute, pallid squamules.

In the phylogenetic analysis, the *M. dolichaula* specimen from Pakistan shared a 100% identity with HM125516 (China) and AF482839 (Australia), 99.7% identity with AY083193 (Australia), and 99.6% identity with HM125517 (China). All analyzed *M. dolichaula* sequences form a sister clade with *M. detersa* (Fig. 3).

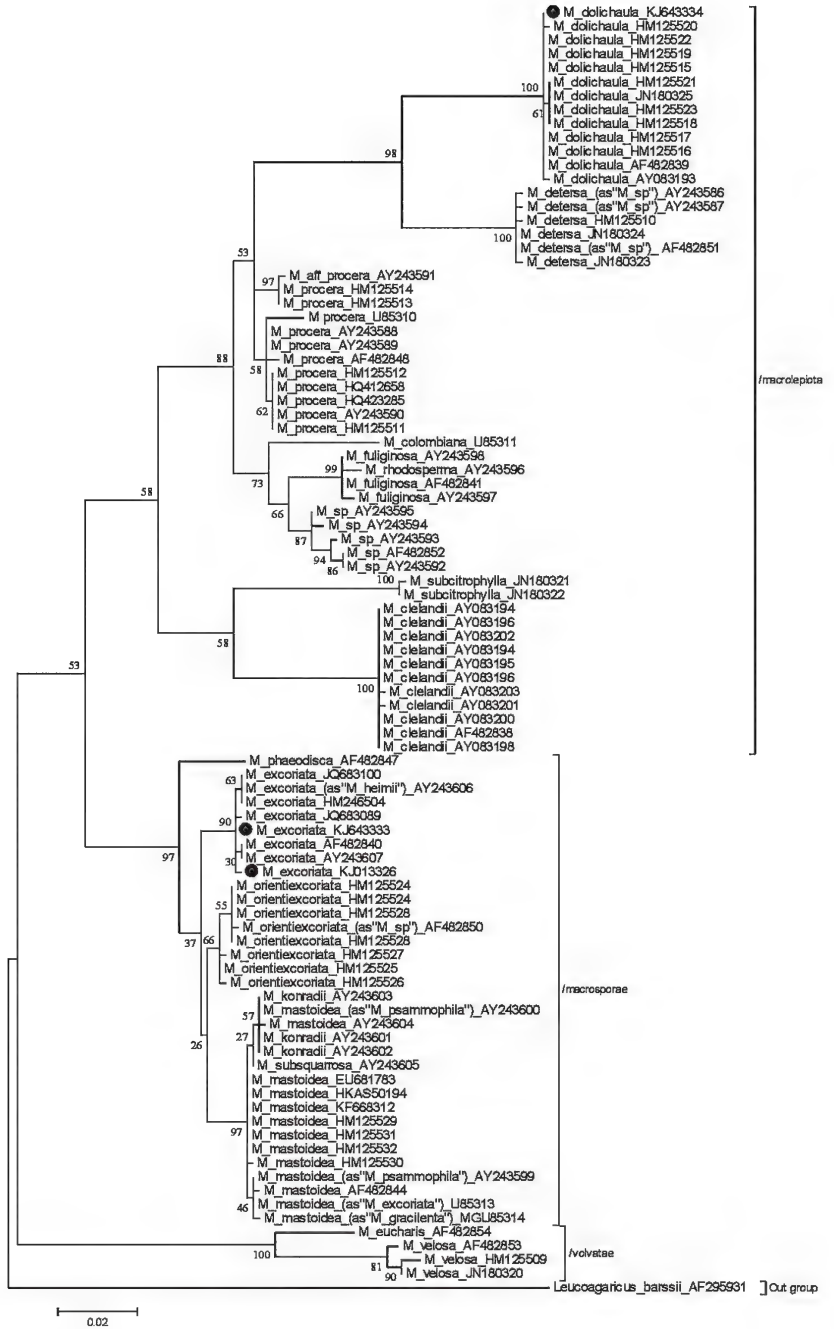

M_dolichaula_KJ643334
M_dolichaula_HM125520
M_dolichaula_HM125522
M_dolichaula_HM125519
M_dolichaula_HM125515
M_dolichaula_HM125521
M_dolichaula_JN180325
M_dolichaula_HM125523
M_dolichaula_HM125518
M_dolichaula_HM125517
M_dolichaula_HM125516
M_dolichaula_AF482839
M_dolichaula_AY083193
M_detersa_(as"M_sp")_AY243586
M_detersa_(as"M_sp")_AY243587
M_detersa_HM125510
M_detersa_JN180324
M_detersa_(as"M_sp")_AF482851
M_detersa_JN180323
M_aff_procera_AY243591
M_procera_HM125514
M_procera_HM125513
M_procera_U85310
M_procera_AY243588
M_procera_AY243589
M_procera_AF482848
M_procera_HM125512
M_procera_HQ412658
M_procera_HQ423285
M_procera_AY243590
M_procera_HM125511
M_colombiana_U85311
M_fuliginosa_AY243596
M_rhodosperma_AY243596
M_fuliginosa_AF482841
M_fuliginosa_AY243597
M_sp_AY243595
M_sp_AY243594
M_sp_AY243593
M_sp_AF482852
M_sp_AY243592
M_subcitrophylla_JN180321
M_subcitrophylla_JN180322
M_clelandii_AY083194
M_clelandii_AY083196
M_clelandii_AY083202
M_clelandii_AY083194
M_clelandii_AY083195
M_clelandii_AY083196
M_clelandii_AY083203
M_clelandii_AY083201
M_clelandii_AY083200
M_clelandii_AF482838
M_clelandii_AY083198
/macrolepiota
M_phaeodisca_AF482847
M_excoriata_JQ683100
M_excoriata_(as"M_heimii")_AY243606
M_excoriata_HM246504
M_excoriata_JQ683089
M_excoriata_KJ643333
M_excoriata_AF482840
M_excoriata_AY243607
M_excoriata_KJ013326
M_orientiexcoriata_HM125524
M_orientiexcoriata_HM125524
M_orientiexcoriata_HM125528
M_orientiexcoriata_(as"M_sp")_AF482850
M_orientiexcoriata_HM125528
M_orientiexcoriata_HM125527
M_orientiexcoriata_HM125525
M_orientiexcoriata_HM125526
M_konradii_AY243603
M_mastoidea_(as"M_psammophila")_AY243600
M_mastoidea_AY243604
M_konradii_AY243601
M_konradii_AY243602
M_subsquarrosa_AY243605
M_mastoidea_EU681783
M_mastoidea_HKAS50194
M_mastoidea_KF668312
M_mastoidea_HM125529
M_mastoidea_HM125531
M_mastoidea_HM125532
M_mastoidea_HM125530
M_mastoidea_(as"M_psammophila")_AY243599
M_mastoidea_AF482844
M_mastoidea_(as"M_excoriata")_U85313
M_mastoidea_(as"M_gracilenta")_MGU85314
/macrosporae
M_eucharis_AF482854
M_velosa_AF482853
M_velosa_HM125509
M_velosa_JN180320
/volvatae
Leucoagaricus_barssii_AF295931
Out group
0.02

FIGURE 3 (left): Molecular phylogenetic analysis of *Macrolepiota* spp. inferred by using the Maximum Likelihood method. Sequences generated from Pakistan are marked with ●. Genbank accession numbers of all the taxa are given. The percentage of trees in which the associated taxa clustered together at 1000 bootstraps is shown next to the branches. The tree is drawn to scale, with branch lengths measured in the number of substitutions per site. The analysis involved 93 nucleotide sequences. There were a total of 712 positions in the final dataset.

Macrolepiota excoriata is closely related to *M. orientiexcoriata* Z.W. Ge et al. but forms a separate subclade within /macrosporae. *Macrolepiota* sect. *Macrosporae* is characterized by a long stipe, a simple annulus, and rare clamp connections. The species within this clade have furfuraceous fine squamules with rarely branched, light brown cylindrical hyphae. *Macrolepiota excoriata* (KJ013326) and *M. excoriata* (KJ643333) from Pakistan shared a 99.9% identity and clustered with *M. excoriata* from Israel (JQ683089, JQ683100, JQ683089), Italy (HM246504), the Netherlands (AF482840), the United Kingdom

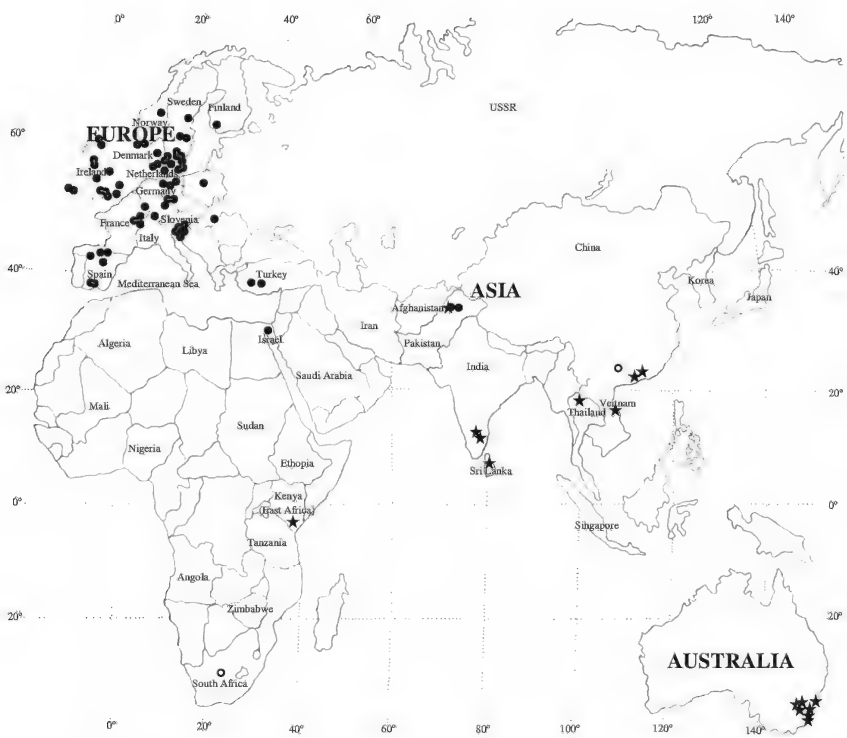


FIGURE 4: Map showing distribution of *Macrolepiota dolichaula* (★) and *M. excoriata* (●). Open dots (○) represent reports of *M. excoriata* without voucher specimens.

(AY243606), and France (AY243607) with 99.7–99.9% identity and 0.2–0.3% genetic divergence.

Many *Macrolepiota* species have relatively small distribution areas (Courtecuisse & Duhem 1994; Nauta & Vellinga 1995; Candusso & Lanzoni 1990). Occurrence of *M. dolichaula* and *M. excoriata* in KPK, Pakistan extends the known distributions of these species considerably (Fig. 4). These two species were found in two different habitats dominated by obligatory mycorrhizal tree species. Mansehra district, KPK, lies in the moist temperate region dominated by *Pinus wallichiana* where the forest floor is dominated by different grass species. These coniferous forests are very similar to those in other parts of the northern temperate zone of Europe and America (Champion et al. 1965). Swat district, KPK, falls in the dry temperate region dominated by *Cedrus deodara* forests, in which there is no understory vegetation.

Although *Macrolepiota* species are generally very regional and are not widely distributed, *M. dolichaula* is an exception. We still lack enough data on the full distribution of these fungi to be able to predict their occurrence (or non-occurrence) in a certain area.

Acknowledgements

We are sincerely thankful to Dr. Else C. Vellinga (University of California, Berkeley, USA) for critically reviewing the manuscript, valuable suggestions to improve the map and acting as presubmission reviewer. Thanks to Dr. Zai-Wei Ge (Kunming Institute of Botany, Chinese Academy of Sciences, P. R. China) for his useful suggestions to improve the manuscript. We are also thankful to Dr. T. K. Arun Kumar (The Zamorin's Guruvayurappan College, India) for acting as presubmission reviewer. We are highly indebted to Higher Education Commission (HEC), Pakistan for financial assistance under Indigenous PhD Fellowships (Phase II).

Literature cited

Afyon A, Yağiz D. 2004. Macrofungi of Sinop Province. Turkish J. Bot. 28: 351–360.

Ahmad S. Iqbal SH, Khalid AN. 1997. Fungi of Pakistan. Sultan Ahmad Mycological Society, Pakistan. 248 p.

Barseghyan GS, Kosakyn A, Isikhuemhen OS, Didukh M, Wasser SP. 2012. Phylogenetic analysis within genera *Morchella* (*Ascomycota*, *Pezizales*) and *Macrolepiota* (*Basidiomycota*, *Agaricales*) inferred from rDNA ITS and EF-1a sequences. JK Misra et al. (eds.). Systematics and evolution of fungi (ed. 1). Science Publishers.

Bi ZS, Zheng GY, Li TH. 1994. Macrofungus flora of Guangdong Province. Guangdong Science and Technology, Guangdong. 879 p. [in Chinese].

Bi ZS, Li TH, Zhang WM, Song B. 1997. A preliminary agaric flora of Hainan Province. Guangdong Higher Education, Guangzhou. 388 p. [in Chinese].

Bon M. 1996. Die Großpilzflora von Europa 3. *Lepiotaceae*. Eching: IHW-Verlag.

Bruns TD. 1995. Thoughts on the processes that maintain local species diversity of ectomycorrhizal fungi. Pl. Soil 170: 63–73. http://dx.doi.org/10.1007/BF02183055

Candusso M, Lanzoni G. 1990. *Lepiota* s.l. Fungi europaei 4. Saronno, Italy: Giovanna Biella. 743 p.

Champion HG, Sethi SK, Khattak GM. 1965. Forest types of Pakistan. Pakistan Forest Institute. Peshawar, Pakistan. 233 p.

Chiu WF. 1948. The *Amanitaceae* of Yunnan. Sci. Rept. Natl. Tsing Hua Univ. Ser. B., Biol. and Psychol. Sci 3(3): 165–178.

Courtecuisse R, Duhem B. 1994. Guide des champignons de France et d'Europe. Lausanne: Delachaux et Niestlé. 478 p.

Ding ZQ, Huang SZ. 2003. Characteristics and high-yield culture technique of *Macrolepiota procera*. Edible Fungi 4: 33 [in Chinese].

Doğan HH, Öztürk, C. 2006. Macrofungi and their distribution in Karaman Province, Turkey. Turk. J. Bot. 30: 193–207.

Doidge EM, 1950. The South African fungi and lichens. Bothalia 5: 1–1094.

Gardes M, Bruns TD. 1993. ITS primers with enhanced specificity for *Basidiomycetes*: application to the identification of mycorrhizae and rusts. Mol. Ecol. 2: 113–118.

Ge ZW, Yang ZL, Vellinga EC. 2010. The genus *Macrolepiota* (*Agaricaceae*, *Basidiomycota*) in China. Fungal Diversity. 45: 81–98. http://dx.doi.org/10.1007/s13225-010-0062-0

Ge ZW, Chen ZH, Yang ZL. 2012. *Macrolepiota subcitrophylla* sp. nov., a new species with yellowish lamellae from southwest China. Mycoscience 53: 284–289. http://dx.doi.org/10.1007/S10267-011-0167-7

Grgurinovic CA. 1997. Larger fungi of South Australia. Botanic Gardens of Adelaide and State Herbarium and Flora and Fauna of South Australia Handbooks Committee, Adelaide.

Kiet TT. 1998. Preliminary checklist of macrofungi of Vietnam. Feddes Repert. 109: 257–277.

Kirk PM, Cannon PF, Minter DW, Stalpers JA. (eds) 2008. Dictionary of the fungi, 10th edn. CABI, Wallingford.

Kumari B. 2012. Diversity, sociobiology and conservation of lepiotoid and termitophilous mushrooms of north west India. PhD Dissertation. Punjabi University, Patiala, India.

Manjula B. 1983. A revised list of the agaricoid and boletoid basidiomycetes from India and Nepal. Proc. Indian Acad. Sci. (Plant Sciences) 92: 81–213.

Mao XL. 2000. The macrofungi in China. Henan Science and Technology, Zhengzhou. 719 p. [in Chinese].

Munsell Soil Color Charts. 1975. Munsell Color Co., Baltimore, MD.

Mustafa G. 2003. Mansehra, an introduction. Gazetteer of the Hazara District.

Natarajan K, Manjuk B. 1983. South Indian *Agaricales* XII – *Lepiota*. Bibliotheca Mycologica 91: 563–581.

Nauta MM, Vellinga EC. 1995. Atlas van Nederlandse paddestoelen. Rotterdam, Brookfield: A.A. Balkema. 352 p.

Pegler DN, Rayner RW. 1969. A contribution to the agaric flora of Kenya. Kew Bulletin 23: 347–412.

Pegler DN. 1977. A preliminary agaric flora of east Africa. Kew Bulletin Additional Series, London 6: 1- 615.

Shah GM, Khan MA. 2006. Check list of medicinal plants of Siran Valley Mansehra-Pakistan, Ethnobotanical Leaflets 10: 63–71.

Shao LP, Xiang CT. 1981. The study on the *Macrolepiota* spp. in China. J. NE Forestry Inst. 4: 35–38.

Singer R. 1948. New and interesting species of *Basidiomycetes*. Pap. Mich. Acad. Sci., Arts Lett. 32: 103–150.

Teng SC. 1996. Fungi of China. Mycotaxon Ltd, Ithaca, New York.

Vellinga EC. 2003. *Chlorophyllum* and *Macrolepiota* (*Agaricaceae*) in Australia. Austr. Syst. Bot. 16: 361–370.

Vellinga EC. 2004. Ecology and distribution of lepiotaceous fungi (*Agaricaceae*) – A Review. Nova Hedwigia 78: 273–299.

Vellinga EC, Kok RPJ de. 2002. Proposal to conserve the name *Chlorophyllum* Massee against *Endoptychum* Czern. Taxon 51: 563–564.

Vellinga EC, Kok RPJ de, Bruns TD. 2003. Phylogeny and taxonomy of *Macrolepiota* (*Agaricaceae*). Mycologia 95: 442–456.

Yang ZL. 2000. Type studies on agarics described by N. Patouillard (and his co-authors) from Vietnam. Mycotaxon 75: 431–476.

Zang M, Li B, Xi JX. 1996. Fungi of Hengduan mountains. Science, Beijing. [in Chinese]

ISSN (print) 0093-4666 © 2014. Mycotaxon, Ltd. ISSN (online) 2154-8889

MYCOTAXON

http://dx.doi.org/10.5248/129.209

Volume 129(1), pp. 209–211 *July–September 2014*

Hyphodontia dhingrae sp. nov. from India

Samita, S.K. Sanyal*, & G.S. Dhingra

Department of Botany, Punjabi University, Patiala 147 002, India

*Correspondence to: *skskumar73l@gmail.com*

Abstract — A new corticioid species with a hydnoid hymenophore, *Hyphodontia dhingrae,* is described in association with an angiospermous host from Uttarakhand state in India.

Key words — *Agaricomycetes,* Bageshwar, Kausani, *Hymenochaetales*, *Schizoporaceae*

While conducting fungal forays in Kausani area of district Bageshwar, Uttarakhand, India, Samita & Sanyal collected an unknown corticioid fungus on angiospermous wood. The study revealed distinct macroscopic and microscopic features such as hydnoid basidiocarps with very long spines (up to 6 mm), two types of cystidial elements, and ellipsoid thin-walled inamyloid basidiospores. Comparison with available literature (Eriksson & Ryvarden 1976, Rattan 1977, Langer 1994, Dhingra 2005, Xiong et al. 2009, 2010, Bernicchia & Gorjón 2010, Gorjón 2012) showed it to be an undescribed species in the genus *Hyphodontia.* The material was also analyzed by Prof. Nils Hallenberg, who supported the description of a new species within *Hyphodontia.*

Hyphodontia dhingrae Samita & Sanyal, **sp. nov.** Figs 1–12

MycoBank MB808470

Differs from *Hyphodontia arguta* by its strongly hydnoid hymenophore and narrower basidiospores.

Type: India, Uttarakhand: Bageshwar, Kausani, on angiospermous wood, 03 September 2011, Samita 5199 (PUN, **holotype**).

Etymology: In honor of Gurpaul Singh Dhingra, Professor, Department of Botany, Punjabi University, Patiala, India.

Basidiocarp resupinate, adnate, effused, ≤300 μm thick in section excluding aculei; hymenial surface cream colored with an orange tint to pale orange when fresh, light orange white to light brownish orange on drying, hydnoid; aculei densely aggregated, cylindrical to conical or slightly flattened, up to 6

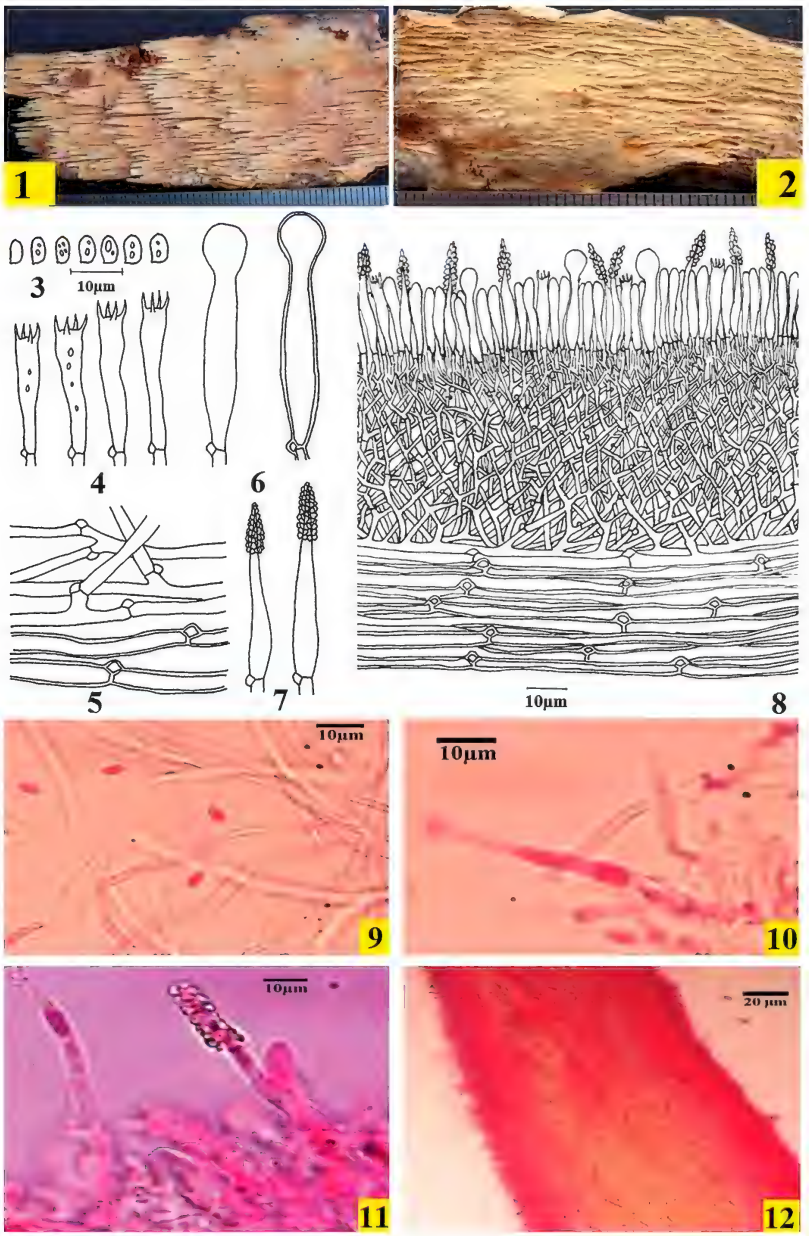


PLATE 1. *Hyphodontia dhingrae* (holotype). 1. Fresh basidiocarp. 2. Dried basidiocarp. 3. Basidiospores. 4. Basidia. 5. Generative hyphae. 6. Capitate cystidia. 7. Lagenocystidia. 8. Vertical section through basidiocarp. 9. Generative hyphae and Basidiospores. 10. Capitate cystidium. 11. Lagenocystidia. 12. Section through spine.

mm long and 1 mm wide at the base; margins thinning, paler concolorous to indeterminate. Hyphal system monomitic; generative hyphae, septate, clamped; basal hyphae ≤5 µm wide, parallel to the substrate, sparsely branched, thin- to somewhat thick-walled; subhymenial hyphae ≤2.5 µm wide, frequently branched and interwoven, thin-walled; tramal hyphae in the centre of the aculei up to 4.5 µm wide, running parallel, densely united, with thickened walls and sparsely septated, without crystalline aggregations. Capitate cystidia 21–47 × 4.5–8 µm scattered in the hymenium, thin- to somewhat thick-walled, with basal clamp. Lagenocystidia 35–40 × 4–6 µm, very frequent in the hymenial region, consisting of hyphal ends abruptly ending into a needle like portion, apically encrusted, thin-walled. Basidia 22–28 × 3.5–4.5 µm, clavate, 4-sterigmate, with basal clamp; sterigmata ≤4 µm long. Basidiospores 4.3–5.3 × 2.5–3 µm, ellipsoid, smooth, thin-walled, with oily contents, acyanophilous, inamyloid.

Remarks— *Hyphodontia dhingrae* is closely related to *H. arguta* in having a similar type of lagenocystidia, but *H. arguta* differs in having an odontioid hymenophore with 0.5–2 mm long spines and wider basidiospores (3.5–5 µm; Eriksson & Ryvarden 1976: 609).

Acknowledgements

The authors thank Head, Department of Botany, Punjabi University, Patiala, for providing research facilities, Prof. Nils Hallenberg (Gothenburg, Sweden) for expert comments and peer review, and Prof. B.M. Sharma (Department of Plant Pathology, COA, CSKHPAU, Palampur, H.P., India) for peer review.

Literature cited

Bernicchia A, Gorjón SP. 2010. *Corticiaceae* s.l. Fungi Europaei 12. Edizioni Candusso. Alassio. Italia. 1008 p.

Dhingra GS. 2005. Genus *Hyphodontia* John Eriksson in the Eastern Himalaya. J. Ind. Bot. Soc. 84: 118–122.

Eriksson J, Ryvarden L. 1976. The *Corticiaceae* of North Europe. Vol. 4. *Hyphodermella – Mycoacia.* Oslo. pp. 549–886.

Gorjón SP. 2012. Some species of *Hyphodontia* s.1. with encrusted cystidial elements. Mycosphere 3(4): 464–474. http://dx.doi.org/10.5943/mycosphere/3/4/10

Langer E. 1994. Die Gattung *Hyphodontia* John Eriksson. Bibliotheca Mycologica 154. 298 p.

Rattan SS. 1977. The resupinate *Aphyllophorales* of the North Western Himalayas. Bibliotheca Mycologica 60. 427 p.

Xiong HX, Dai YC, Wu SH. 2009. Three new species of *Hyphodontia* from Taiwan. Mycol. Progress 8: 165–169. http://dx.doi.org/10.1007/s11557-009-0587-3

Xiong HX, Dai YC, Wu SH. 2010. Two new species of *Hyphodontia* from China. Mycologia 102(4): 918–922. http://dx.doi.org/10.3852/09-139

ISSN (print) 0093-4666 © 2014 Mycotaxon, Ltd. ISSN (online) 2154-8889

MYCOTAXON

http://dx.doi.org/10.5248/129.213

Volume 129(1), pp. 213–214 *July–October 2014*

Nomenclatural novelties and typifications proposed in Mycotaxon 129(1)

Agaricus taeniatus Sai F. Li, Shao J. Li & H.A. Wen, p. 189
Aschersonia narathiwatensis Mongkols., Khonsanit & Luangsa-ard, p. 36
Erysiphe magnoliicola S.E. Cho, S. Takam. & H.D. Shin, p. 155
Gastroboletus thibetanus Shu R. Wang & Yu Li, p. 80
Hyphodontia dhingrae Samita & Sanyal, p. 209
Hypoxylon confertisilvae Lar.N. Vassiljeva & S.L. Stephenson, p. 90
Hypoxylon ilicinum Lar.N. Vassiljeva & S.L. Stephenson, p. 92
Hypoxylon meridionale Lar.N. Vassiljeva & S.L. Stephenson, p. 92
Hypoxylon minicroceum Lar.N. Vassiljeva & S.L. Stephenson, p. 93
Hypoxylon rolingii Lar.N. Vassiljeva & S.L. Stephenson, p. 94
Linkosia longirostrata G. Delgado, p. 42
Nawawia quadrisetulata Goh, W.Y. Lau & K.C. Teo, p. 110
Neofomitella Y.C. Dai, Hai J. Li & Vlasák, p. 12
Neofomitella fumosipora (Corner) Y.C. Dai, Hai J. Li & Vlasák, p. 12
Neofomitella polyzonata Y.C. Dai, Hai J. Li & Vlasák, p. 12
Neofomitella rhodophaea (Lév.) Y.C. Dai, Hai J. Li & Vlasák, p. 15
Nolanea acuta (Romagn. & Gilles) Largent, p. 121
Nolanea alboproxima Largent, Aime & T.W. Henkel, p. 123
Nolanea applanata Largent & T.W. Henkel, p.125
Nolanea clavata Largent & T.W. Henkel, p. 127
Nolanea claviformis Largent & Aime, p. 130
Nolanea concentrica Largent & T.W. Henkel, p. 132
Nolanea furcata Largent & T. W. Henkel, p. 134
Nolanea mimiae Largent & Aime, p. 137
Nolanea rava Largent & Aime, p. 139
Nolanea sinuolata Largent, Aime, & T.W. Henkel, p. 141
Nolanea subsulcata Largent & T.W. Henkel, p. 144
Phoma recepii Halıcı & Candan, p. 164

Rhizophagus natalensis Błaszk., Chwat & B.T. Goto, p. 100
Uromyces myosotidis Bahç., p. 21
Xylaria beilschmiediae G. Huang & L. Guo, p. 149
Xylaria foliicola G. Huang & L. Guo, p. 150
Ypsilomyces D.A.C. Almeida & Gusmão, p. 182
Ypsilomyces elegans D.A.C. Almeida & Gusmão, p. 182
Zasmidium gynurae (Sawada & Katsuki) W.H. Hsieh, Y.L. Guo & F.Y. Zhai, p. 57
Zasmidium bougainvilleae (J.M. Yen & G. Lim) Y.L. Guo, W.H. Hsieh & F.Y. Zhai, p. 58

bad taxonomy

can KILL